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LITHIUM ACTION ON GLUCOSE UPTAKE IN BRAIN; ROLE OF GLUCOSE-1,6-P_2 AS A REGULATOR OF HEXOKINASE

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SUMMARY

When given intraperitoneally to mice, lithium chloride decreased α-glucose-1,6-P_2 in the brain to about 30% of normal. This may explain the observation that Li^+ stimulates glucose utilization by brain and other tissues insofar as α-glucose-1,6-P_2 inhibits animal hexokinase strongly. Glucose-1,6-P_2 synthase activity of brain was much lower in Li^+-animals when assayed without added divalent metal co-factor such as Mg^{2+} but the same with Mg^{2+} in the assay. This results because Li^+ replaces the tightly bound activator, probably Zn^{2+}. These results demonstrate the importance of α-glucose-1,6-P_2 in regulation of hexokinase and suggest that normal energy metabolism of the brain may readily become sensitive to control by metal ion concentration.

Salts of lithium ion, administered intraperitoneally to the mouse have been reported to give increased glucose utilization of brain, diaphragm, and liver (1-4). Preparations of skeletal muscle (5-8) and brain slices (9) show significant stimulation of glucose uptake when Li^+ is included in the incubation at a small fraction of the Na^+ concentration. The effects on brain, in which the transport of glucose into the cell is rapid, implies an activating effect by Li^+ at the hexokinase step either by direct action on the enzyme or indirectly through control of metabolites that influence the hexokinase rate. With diaphragm Li^+ (25 mM) was reported to stimulate both glycogen synthesis and the activity of glycogen synthase (8). A 40% lowering of glucose-6-P concentration was observed in this system (7) suggesting one mechanism for indirect control of hexokinase.

A second, indirect effect of Li^+ on glucose utilization rate might be the consequence of its inhibition of glucose-1,6-P_2 synthase (10): α-Glc-1-P (or Glc-6-P) + Glycerate-1,3-P_2 → α-Glc-1,6-P_2 + Glycerate-3-P. Glucose-1,6-P_2 is a good inhibitor of hexokinase (11-13) even stronger than glucose-6-P for type II hexokinase below about pH 7.3 where they are about equivalent (14). Type I hexokinase of red blood cells (15) and Sarcoma 37 cells (14) may be somewhat less

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inhibited by glucose-1,6-P_2 (12, 13, 15) but a report that it is not an inhibitor of isozyme I (13) probably reflects the pH effect. In the physiological range of pH the K_i values are well below the concentrations of glucose-1,6-P_2 found in red cells (16) brain and other tissues (17).

The present study with mice given LiCl finds the brain synthase to be in an inhibited state and the glucose-1,6-P_2 level to be greatly depressed, consistent with the hypothesis that Li^+ may increase glucose utilization as proposed.

Effect of Li^+ on Glc-1,6-P_2 of Mouse Brain. Following the procedure of De Feudis (4) NaCl or LiCl, at 7 mmoles/kgm, was injected intraperitoneally into 8 week old ICR female albino mice, ∿ 30 g. Three hours later the heads were removed and rapidly stirred in Freon-12 at -150°. Subzero dissection and powdering, followed by homogenization in methanol-HCl and precipitation with $HClO_4$ was by the procedure of Lowry and Passonneau (18, 19). The cerebellum and medulla were excluded. The level of Glc-1,6-P_2 in Li^+ injected mice was only 20-30% of that found in the control, Table I. Glc-6-P decreased significantly as well. Both Glc-6-P and Glc-1,6-P_2 are known to fall after decapitation unless freezing is rapid and dissection done at very low temperatures. This artifact would result in a 2-3x increase in fructose-1,6-P_2, due to the anaerobic condition, however (18).

Table I

Effect of Li^+ on Brain Metabolites

	Control, Na^+ Mouse	Literature Values (17, 18)	Lithium Mouse 1	Lithium Mouse 2
		(mμmoles/gm wet wt)		
Glc-1,6-P_2[a]	68	72 ± 5	16	19
Glucose-6-P[b]	40	65 ± 6	23	29
Fructose-1,6-P_2[c]	132	120	84	100

[a] Samples containing < 10^{-8} moles Glc-1,6-P_2 were incubated in 1 ml containing triethanolamine-HCl (pH 7.5, 100 mM), Glc-1-P (0.1 mM), EDTA (0.5 mM), $MgCl_2$ (3 mM), DTT (1 mM) and dephospho-phosphoglucomutase (10 munits). After 30' at 25° Glc-6-P was assayed with Glc-6-P dehydrogenase. Assay was proportional to sample size and gave the expected response to internal standards.

[b] Assayed spectrophotometrically by use of glucose-6-P dehydrogenase and TPN^+.

[c] Assayed with a mixture of aldolase, triose P isomerase, glycerol-P dehydrogenase and DPNH.

Effect of Li^+ on Glc-1,6-P_2 Synthase. The large effect of Li^+ on Glc-1,6-P_2 was expected from earlier studies with purified Glc-1,6-P_2 synthase (10) showing ia K_i of Li^+ of about 50 μM in competition with Mg^{2+}, $K_m \cong 500$ μM. However, fresh brain homogenates were partly resistant to inhibition by EDTA or Li^+. This resistant activity was proposed to represent the presence of tightly bound metal activators such as Zn^{2+}. That this is indeed the explanation for the EDTA resistant activity of brain is shown in Figure 1. The Mg^{2+}-independent activity of brain extract containing EDTA was followed as a function of time and temperature of incubation. At 25° and 37° activity was lost with a half time of 15 min and 5 min, respectively. Glc-1,6-P_2 which results in phosphorylation of the synthase (21) did

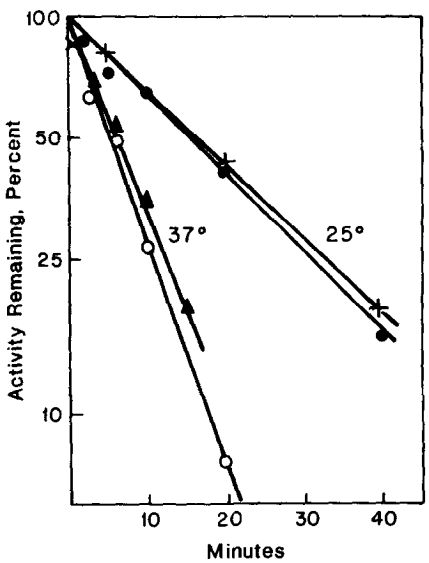

Figure 1. Loss of synthase activity of fresh brain homogenate in EDTA at 25° and 37°. Synthase activity was assayed in 0.1 ml (10): triethanolamine-HCl (0.1 M, pH 7.8), glycerate-[1-^{32}P]-1,3-P_2 (1.5 μM, ~ 10^5 cpm), EDTA (1 mM) and Glc-1-P (0.5 mM). After incubation at 25° for 4' with added brain extract the reaction was terminated with H_2SO_4 (400 μmoles) and P_i (0.2 μmoles) added in 0.3 ml and the tube heated to 100° for 10' to hydrolyze any acid labile ^{32}P compound. The $^{32}P_i$ was extracted with molybdate and isobutanol (20) leaving counts remaining in the aqueous phase as a measure of Glc-1,6-P_2 formed. Activity in the absence of Glc-1-P was also measured and subtracted. Rates were linear with time during the assay. Enzyme samples were prepared as follows: a single mouse brain was homogenized at 4° in 2 ml of 0.25 M sucrose, triethanolamine acetate (pH 8.0, 10 mM) and EDTA (10 mM). The enzyme containing supernatant fraction was prepared by centrifugation at 10,000 x g, 10 min. Loss of activity was measured using a 1/10 dilution in the same medium and incubating it at 25° (●-●) or 37° (○-○) as shown. Zn^{2+} was restored to enzyme after 30 min. incubation at 37° by passage through a G-25 Sephadex column equilibrated with 10 mM triethanolamine acetate pH 8.0 and 0.5 mM $ZnCl_2$ and stability in 10 mM EDTA determined again at 25° (X-X). The effect of Glc-1,6-P_2, 50 μM, on stability of native enzyme at 37° was also measured (▲-▲).

Table II

Li^+ Inhibition of Synthase in Fresh and Aged Brain Extract; Effect of Zn^{2+}

Enzyme[a]	-Mg		+Mg	
	-Li	+Li	-Li	+Li
	(munits/g wet wt of brain)			
Fresh Extract	205	193	330	176
Aged Extract	11	9	269	11
Aged + Zn^{2+}	188	192	286	184

[a] The three enzyme samples were prepared and assayed as in Figure 1 with additions of Li^+ (2.5 mM) and Mg^{2+} (5 mM) to the assay as noted.

not alter the decay rate. Activity could be recovered if Zn^{2+} was exchanged for EDTA, Figure 1. Subsequent addition of EDTA caused loss of activity at 25° at the expected rate if Zn^{2+} was the responsible endogenous activator.

Table II shows the insensitivity to Li^+ of the activity of fresh homogenate. The activity dependent on the addition of Mg^{2+} was fully inhibited by Li^+ unlike the activity restored by Zn^{2+}. As did the experiments with EDTA these results reflect the slow dissociation of Zn^{2+} from the enzyme and the rapid dissociation of Mg^{2+}.

Effect of Li^+ on the State of Synthetase In Vivo. Inhibition by Li^+ of synthase activity in vivo should depend on the levels of both the rapid- and slow-dissociating metal ions. Table II shows that about 65% of the fresh brain homogenate activity is Mg^{2+}-independent and Li^+ insensitive. The fact that this is not 100% means that a rapidly dissociating metal ion such as Mg^{2+} is a successful competitor with Zn^{2+} in the brain. This makes it likely that Li^+ will also be a competitive factor in vivo.

As reported in Table III the brain extract of the Li^+ treated animal expressed only $\sim$ 16% of its activity in the absence of Mg^{2+} compared with 59% in the control extract. With Mg^{2+} in the assay the rates were the same. This result was confirmed

Table III

Effect of Li^+ Injection on Brain Synthase

Brain homogenate assayed[a] Salt injected	Wet wt brain assayed	Synthase Rate[b]: Counts in Glc-1,6-P_2 -Mg	+Mg (5 mM)	Mg Independent
	µg	cpm		percent
NaCl	10	3132	5332	59
LiCl	20	1800	10,965	16
Combined	10 + 20	4642	15,730	30
Expected for combined		4932	16,297	30

[a] Either NaCl or LiCl, 7 mmoles/kg, was injected 3 h before obtaining the brain which was dissected in the coldroom and homogenized and centrifuges as in Figure 1.

[b] Assayed as in Figure 1.

in several experiments at both 3 and 6 h after Li^+/Na^+ injection. The combination of brain extracts from control and Li^+ animals are seen to give additive results indicating the absence of secondary inhibitory factors in the less active extract at the dilution used in the assay.

DISCUSSION In addition to its inhibitory effect on hexokinase, Glc-1,6-P activates phosphofructokinase of brain, muscle, and red cell (22, 24). Under conditions where this is operative, a decrease in Glc-1,6-P_2 would therefore have the effect of shunting extra glucose to glycogen. This is seen in experiments by Haugaard et a. (7) with isolated rat diaphragm treated with Zn^{2+}. In brain where the high level of fructose-1,6-P_2, Table I, would dampen any influence of a change in Glc-1,6-P_2 on phosphofructokinase, a change in partition of Glc-6-P might reflect only the activation of glycogen synthase by Li^+ (8).

Glc-1,6-P_2 of human red blood cells is insensitive to Li^+. This was shown by incubating cells for 17 hours in 25 mM Li^+ (unpublished). Cells from patients receiving Li^+ therapy for 2-3 months were similar to normals in this respect (unpublished results with blood samples kindly provided by J. Mendels). The synthase

of red cells, an isoenzyme of the brain (10) and liver synthases (25) may be insensitive to Li^+.

The role of Glc-1,6-P_2 as a regulatory compound, in the sense of cAMP, may be inferred from its presence in some tissues at concentrations well in excess of its requirement as a coenzyme for phosphoglucomutase, and from its control effects on several key enzymes of energy metabolism, the most recently discovered being pyruvate kinase (26) and phosphogluconate dehydrogenase (27). All regulatory effects occur in the range 10^{-5}-10^{-4} M, which is the range of concentrations seen in muscle, brain and red cells (16, 17). The synthase is inhibited by a number of "regulatory" compounds such as citrate and fructose-1,6-P_2 (10) and there must be an equally active degradative process in brain in view of the short half life of Glc-1,6-P_2 in this tissue, < 1.7' (17). Direct evidence for a regulatory action in vivo has not been available. Taken together with earlier studies that show stimulation by Li^+ of glucose utilization in brain the present study may provide such evidence. Furthermore the present study implicates Zn^{2+} as a factor of possible significance in the control of the brain's utilization of its primary energy source, glucose.

Although it is not meant to suggest that the effect of Li^+ on glucose metabolism is responsible for the Li^+ action in treatment of manic-depression, the present observations suggest a parameter that may be useful in testing its real mode of action. A test of whether Li^+ behaves by altering a Zn^{2+} dependent enzyme such as Glc-1,6-P_2 synthase would be to relate its effectiveness in therapy to the level of Zn^{2+} in the diet.

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