Biochemical Pharmacology, Vol 31, No. 6, pp. 1025–1031, 1982.
Printed in Great Britain.

0006-2952/82/061025-07 $03.00/0

EFFECTS OF LITHIUM ON THE ACTIVITIES OF PHOSPHOFRUCTOKINASE AND PHOSPHOGLUCOMUTASE AND ON GLUCOSE-1,6-DIPHOSPHATE LEVELS IN RAT MUSCLES, BRAIN AND LIVER

JARDENA NORDENBERG, MIRIAM KAPLANSKY, EINAT BEERY, SARAH KLEIN and RIVKA BEITNER*

Department of Life Sciences, Bar-Ilan University, Ramat-Gan 52 100, Israel

(*Received* 20 *July* 1981; *accepted* 9 *October* 1981)

Abstract—Incubation of rat diaphragm muscle in the presence of lithium chloride (a drug used widely in the therapy of patients with mental illness), resulted in a sharp decrease in the level of glucose-1,6-diphosphate (Glc-1,6-P_2), the powerful regulator of carbohydrate metabolism. This decrease in Glc-1,6-P_2, the most potent activator of phosphofructokinase and phosphoglucomutase, was accompanied by a marked reduction in the activities of both enzymes, when assayed in the absence of exogenous Glc-1,6-P_2 under conditions in which these enzymes are sensitive to regulation by endogenous Glc-1,6-P_2. A decrease in Glc-1,6-P_2 and the concomitant reduction in the activities of phosphofructokinase and phosphoglucomutase, were also obtained in the rat gastrocnemius and tibialis anterior muscles, as well as in brain, following Li^+ injection. In contrast to its effects in muscles and brain, Li^+ did not exert any effect on Glc-1,6-P_2 level and on the enzymes' activities in the liver. The marked inhibition of brain and muscles phosphofructokinase (the rate-limiting enzyme in glycolysis) induced by Li^+, may play an important role in the mechanism of the therapeutic action of this agent in the manic state.

Previous experiments from this laboratory have revealed that glucose-1,6-diphosphate (Glc-1,6-P_2) plays a highly important role in the regulation of glycolysis and other pathways of glucose metabolism (for review, see ref. 1). Glc-1,6-P_2 was shown to be one of the strongest activators (deinhibitors) of phosphofructokinase (the rate-limiting enzyme of glycolysis) from many tissues [2–6]. Glc-1,6-P_2 is also an activator of phosphoglucomutase [7], and we have found, that similarly to its deinhibitory action on phosphofructokinase, Glc-1,6-P_2 releases phosphoglucomutase from inhibition by ATP or citrate [8]. Our experiments have revealed that the concentration of Glc-1,6-P_2 in muscle changes under many physiological and pathological conditions, leading to concomitant changes in the activities of phosphofructokinase and of phosphoglucomutase, which are modulated by this regulator [1].

Recently it has been reported that injections of lithium, a drug used widely in the therapy of manic depression, induced a sharp decrease in Glc-1,6-P_2 level in brains of mice [9]. Many investigators have reported that Li^+ exerts effects on glucose metabolism in brain and certain other tissues. Lithium was shown to enhance glucose uptake in brain and muscle [10–12] and to increase glycogen content in these tissues [11, 13, 14], whereas in liver it decreases the concentration of glycogen [14]. It was also reported that Li^+ decreases lactate formation in muscle [11], which may indirectly suggest that it inhibits muscle glycolysis. It has been postulated that Li^+ may affect glucose metabolism at several sites [15]. However, the mechanism of Li^+ action is still unknown.

In an attempt to gain insight into the mechanism of Li^+ action on glucose metabolism, we examined in the present experiments, whether Li^+ exerts an effect on the activity of phosphofructokinase, the key enzyme of glycolysis. The experiments were conducted *in vitro*, in the isolated rat diaphragm, as well as *in vivo*, in gastrocnemius and tibialis anterior muscles, brains and livers of lithium-treated rats. We also examined the effect of Li^+ on the activity of phosphoglucomutase and on the levels of Glc-1,6-P_2, the most potent activator of both enzymes.

MATERIALS AND METHODS

Enzymes and chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). LiCl was obtained from Merck (Darmstadt, F.D.R.). Glucose 1-phosphate was essentially free of glucose-1,6-diphosphate. Imidazole was the low fluorescence grade.

Male Charles River albino rats weighing 70–110 g fed *ad lib.* were used. For *in vitro* studies rats were anesthetized with sodium nembutal (60 mg/kg). Diaphragms were quickly removed and prepared for incubation as described previously [16]. Hemidiaphragms were incubated for 90 min in the absence or presence of LiCl in Krebs–Ringer bicarbonate buffer gassed with 95% O_2–5% CO_2 at 37°. The concentration of glucose in the medium was 1 mg/ml.

* Author to whom all correspondence should be addressed.

Table 1. The effect of LiCl on Glc-1,6-P_2 level in the isolated rat diaphragm

	Glc-1,6-P_2 (μmoles/kg wet wt)	% Effect	P value
Control	6.75 ± 0.30 (6)		
Li^+	4.05 ± 0.38 (6)	−40	<0.00005

Hemidiaphragms were incubated in the absence or presence of 250 μM LiCl for 90 min. Glc-1,6-P_2 was extracted and measured as described in Materials and Methods.

Values are means ± S.E.M. and number of experiments in parentheses.

For *in vivo* experiments rats were injected with 0.1 ml aqueous solution of LiCl at 7 mmoles/kg. Control animals received 0.1 ml of H_2O. Three hours later the tissues were quickly removed and immediately frozen between a pair of aluminium tongs precooled in liquid N_2. Livers were cut and rapidly rinsed in ice cold Krebs–Ringer bicarbonate buffer and then frozen. Frozen tissues were used for Glc-1,6-P_2 and enzyme extractions.

Extraction and determination of Glc-1,6-P_2. The frozen tissues were powdered in a mortar cooled in liquid N_2, and the powder (70–90 mg for muscles and liver and 50–70 mg for brain) was placed on top of 1 ml (for muscles and liver) or 2 ml (for brain) 5% perchloric acid containing 1 mM EDTA, and extracted at −10°. After centrifugation the extract was neutralized with KOH. The precipitated potassium perchlorate was removed by centrifugation and the clear supernatant was used for determination of Glc-1,6-P_2. Glc-1,6-P_2 was estimated by measuring fluorometrically the appearance of NADPH upon addition of glucose-1-phosphate, phosphoglucomutase, glucose-6-phosphate dehydrogenase and $NADP^+$ [17].

Extraction and determination of enzymes. Phosphofructokinase and phosphoglucomutase were extracted and the regulatory and maximal activity of both enzymes were assayed as described previously [18–20]. Protein was measured by the method of Lowry *et al.* [21] with crystalline bovine serum albumin as a standard. The principle of the assay of phosphofructokinase was similar to that of Mansour [22, 23]. The enzyme was coupled with aldolase, triosephosphate isomerase, and α-glycerophosphate dehydrogenase. The rate of NADH oxidation was determined in Zeiss spectrophotometer. One mUnit of phosphofructokinase activity catalysed the formation of 1 nmole of fructose-1,6-P_2 per min at 25°. In the assay of phosphoglucomutase the enzyme was coupled with glucose-6-phosphate dehydrogenase. The rate of NADPH formation was measured in a Zeiss spectrophotometer. One mUnit of phosphoglucomutase activity catalysed the reduction of 1 nmole of $NADP^+$ per min at 25°.

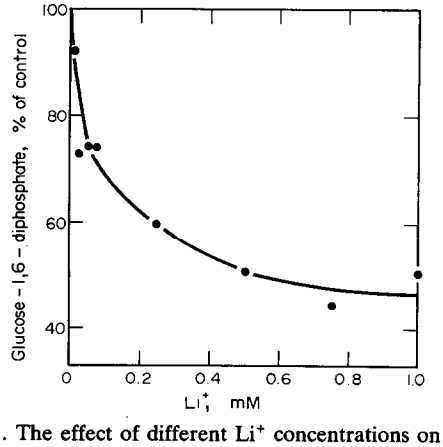

Fig. 1. The effect of different Li^+ concentrations on Glc-1,6-P_2 levels in the isolated rat diaphragm. For each experiment hemidiaphragms from rats were incubated in the absence or presence of different concentrations of LiCl for 90 min. Glc-1,6-P_2 was extracted and measured as described in Materials and Methods.

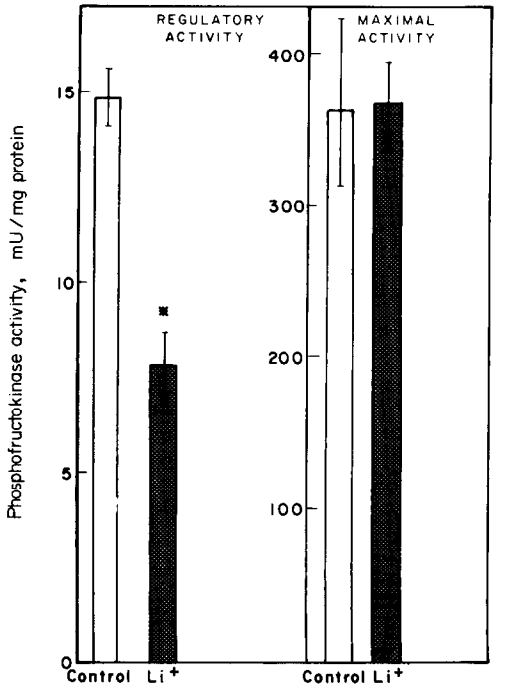

Fig. 2. The effect of Li^+ on the regulatory and maximal activities of phosphofructokinase in the isolated rat diaphragm. Pooled hemidiaphragms of two rats were incubated in the absence and presence of 250 μM Li^+ for 90 min. Phosphofructokinase was extracted and assayed as described in Materials and Methods. Values are means ± S.E.M. for 5 experiments. *$P < 0.0001$; maximal activity: N.S.

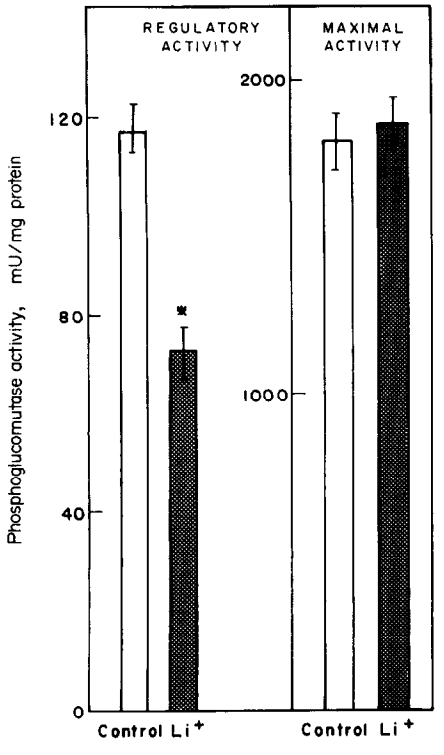

Fig. 3. The influence of Li^+ on the regulatory and maximal activities of phosphoglucomutase in the isolated rat diaphragm. Hemidiaphragms were incubated as described in Fig. 2. Phosphoglucomutase was extracted and assayed as described in Materials and Methods. Values are means ± S.E.M. for 6 experiments. *$P < 0.005$; maximal activity: N.S.

RESULTS

The results presented in Table 1 reveal that incubation of rat diaphragm muscles in the presence of Li^+ resulted in a significant decrease in the concentration of Glc-1,6-P_2. Figure 1 shows that the decrease in Glc-1,6-P_2 could be obtained with extremely low concentrations of Li^+ and was concentration-dependent. As Glc-1,6-P_2 is a most potent activator of phosphofructokinase, the rate-limiting enzyme in glycolysis, we examined the effect of Li^+ on the activity of this enzyme in the isolated rat diaphragm. Figure 2 shows that concomitant to the decrease in Glc-1,6-P_2, lithium exerted a marked decrease in the regulatory activity of phosphofructokinase, causing no change in its maximal activity. The regulatory activity was measured at pH 6.9 under conditions in which the enzyme was subject to regulation by Glc-1,6-P_2 and its other allosteric modulators [19, 22–24]. Maximal activity was measured at pH 8.2, under optimal conditions, where the enzyme shows no regulatory (allosteric) properties [19, 22–24]. The maximal activity reflects the full catalytic potential of the enzyme and the fact that it was not affected by Li^+ indicates that there was no decrease in the total protein content of the enzyme in the cell. The results presented in Fig. 3 show the effect of Li^+ on phosphoglucomutase activity in the isolated rat diaphragm. Similarly to phosphofructokinase, Li^+ induced a very significant decrease in the regulatory activity of this enzyme. Glc-1,6-P_2 was not added to the reaction mixtures of phosphoglucomutase and phosphofructokinase when assayed under regulatory conditions, therefore these enzymes were exposed only to regulation by endogenous Glc-1,6-P_2.

The maximal activity of phosphoglucomutase (measured under optimal conditions, in the presence of saturating concentrations of Glc-1,6-P_2), was not changed by Li^+. In the experiments presented in Table 2 and Figs. 4 and 5, we examined the effects of Li^+ on Glc-1,6-P_2 level and on the activities of phosphofructokinase and phosphoglucomutase in intact rats, following a single Li^+ injection. The effects of Li^+ were tested in brains, livers and in two kinds of skeletal muscles: the gastrocnemius muscle, which represents a mixed muscle of white and red fibers and the tibialis anterior muscle, which is classified as a white glycolytic muscle. The results in Table 2 show a striking reduction in Glc-1,6-P_2 level in both kinds of muscles, as well as in brains of rats following Li^+ injection. The effect induced by Li^+ in the tibialis anterior muscle was slightly more pronounced than that induced in the gastrocnemius muscle. In contrast to its effects in muscles and brain, Li^+ did not affect Glc-1,6-P_2 concentrations in livers of these rats. The decrease in Glc-1,6-P_2 level in brain and muscles induced by Li^+, was accompanied by a marked decrease in the regulatory activities of phosphofructokinase (Fig. 4) and phosphogluco-

Table 2. The effect of Li^+ treatment on Glc-1,6-P_2 levels in muscles, brains and livers of rats

Tissue	Glc-1,6-P_2 (μmoles/kg wet wt) Control	Li^+	% Effect
Tibialis anterior muscle	19.4 ± 1.9	7.1 ± 0.7	−63*
Gastrocnemius muscle	12.9 ± 0.7	6.2 ± 0.4	−52*
Brain	63.6 ± 6.8	28.0 ± 2.6	−56*
Liver	8.8 ± 0.9	9.0 ± 0.6	N.S.

Rats were treated with Li^+ and Glc-1,6-P_2 was extracted and measured as described under Materials and Methods.

Values are means ± S.E.M. for 6–10 experiments.

*$P < 0.005$; N.S. = not significant.

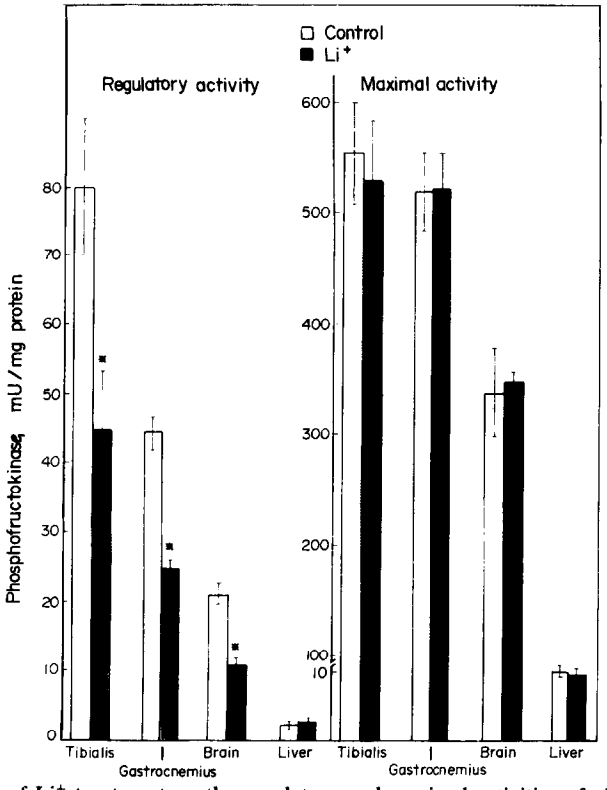

Fig. 4. The effect of Li^+ treatment on the regulatory and maximal activities of phosphofructokinase from tibialis anterior and gastrocnemius muscles, brains and livers of rats. Rats were treated with Li^+ and phosphofructokinase was extracted and assayed as described in Materials and Methods. Values are means ± S.E.M. for 6–8 experiments. *$P < 0.005$ relative to control tissues; other changes not significant.

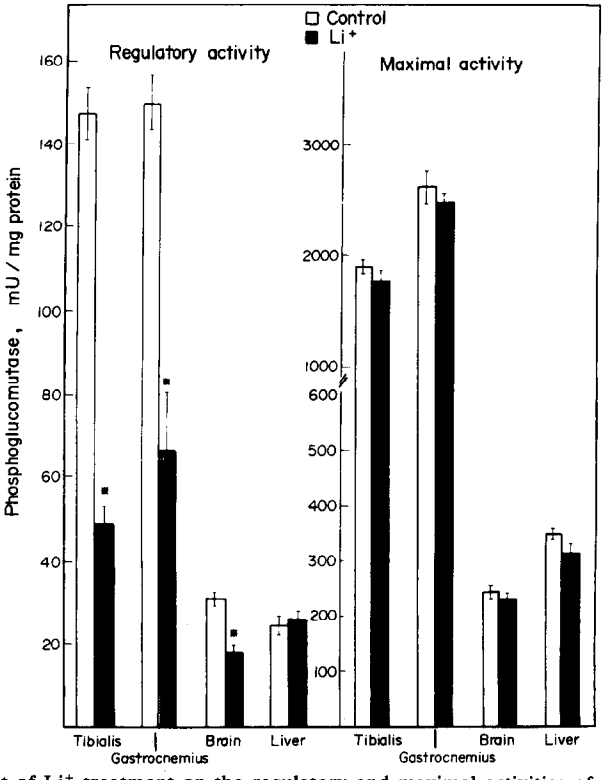

Fig. 5. The effect of Li^+ treatment on the regulatory and maximal activities of phosphoglucomutase from tibialis anterior and gastrocnemius muscles, brains and livers of rats. Rats were treated with Li^+ and phospholgucomutase was extracted and assayed as described in Materials and Methods. Values are means ± S.E.M. for 5–10 experiments. *$P < 0.005$ relative to control tissues; other changes not significant.

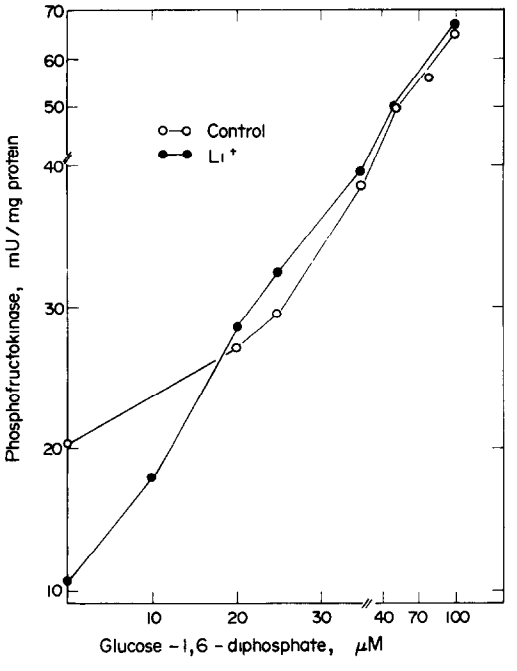

Fig. 6. The activatory effect of Glc-1,6-P_2 on phosphofructokinase from brains of Li^+-treated and untreated rats. Phosphofructokinase was prepared from brains of controls or Li^+-treated rats and assayed under regulatory conditions (pH = 6.9) as described in Materials and Methods. Assays were carried out in the presence of variable Glc-1,6-P_2 concentrations.

mutase (Fig. 5) which are regulated by Glc-1,6-P_2. The maximal activities of both enzymes were not affected by Li^+, indicating that the reduction in the enzymes' activities was due to regulation and not due to alterations in the total content of the enzymes in the cell. In liver, where Glc-1,6-P_2 level was not affected by Li^+, there was also no effect of Li^+ on the activities of phosphofructokinase (Fig. 4) or phosphoglucomutase (Fig. 5).

In the experiments demonstrated in Fig. 6 we examined the effect of Glc-1,6-P_2 on the activity on phosphofructokinase extracted from brains of Li^+-treated or untreated control rats. It can be seen that the brain enzyme (assayed under regulatory conditions) was sensitive to activation by Glc-1,6-P_2 at the physiological concentration range found in brain. The enzyme extracted from brains of Li^+-treated animals was more sensitive to activation by Glc-1,6-P_2 as compared to untreated controls. By adding Glc-1,6-P_2 to the assay mixture of phosphofructokinase extracted from brains of the Li^+-treated rats the inhibitory effect of Li^+ on phosphofructokinase activity was abolished. Table 3 shows that similarly to the findings in brain, when phosphofructokinase from tibialis anterior or gastrocnemius muscles was assayed in the presence of Glc-1,6-P_2, the inhibitory effect of the Li^+ was abolished. The enzyme extracted from muscles of the Li^+-treated animals was more sensitive to activation by Glc-1,6-P_2 as compared to the enzyme from untreated controls. Phosphoglucomutase from the muscles and brain (Table 4) showed a similar behavior to that of phosphofructokinase (Table 3).

Table 3. The activatory effect of Glc-1,6-P_2 on phosphofructokinase from muscles of untreated (controls) and Li^+-treated rats

	Phosphofructokinase (mU/mg protein)			
	Tibialis anterior muscle		Gastrocnemius muscle	
Additions	Control	Li^+	Control	Li^+
None	90.4 ± 11.7	56.0 ± 6.7	45.2 ± 2.4	25.2 ± 1.3
Glc-1,6-P_2 (20 μM)	243.2 ± 19.0	239.6 ± 19.6	94.0 ± 10.0	87.6 ± 10.6
Effect of Glc-1,6-P_2 (%)	+145*	+345*	+110*	+251*

Phosphofructokinase was prepared from muscles of controls or Li^+-treated rats and assayed under regulatory conditions in the absence and presence of Glc-1,6-P_2.
Values are means ± S.E.M. for 6 experiments.
* $P < 0.005$.

Table 4. The activatory effect of Glc-1,6-P_2 on phosphoglucomutase from muscles and brains of untreated (controls) and Li^+-treated rats

	Phosphoglucomutase (mU/mg protein)					
	Tibialis		Gastrocnemius		Brain	
Additions	Control	Li^+	Control	Li^+	Control	Li^+
None	152 ± 8	49 ± 5	140 ± 5	79 ± 5	32 ± 1	18 ± 2
Glc-1,6-P_2 (10 μM)	273 ± 14	356 ± 23	438 ± 49	559 ± 39	91 ± 5	82 ± 6
Effect of Glc-1,6-P_2 (%)	+80*	+620*	+213*	+609*	+182*	+345*

Phosphoglucomutase was prepared from muscles and brains of controls and Li^+-treated rats and measured under regulatory conditions in the absence and presence of Glc-1,6-P_2.
Values are means ± S.E.M. for 5 experiments.
* $P < 0.005$.

DISCUSSION

The results presented here show that lithium exerts a marked inhibition of brain and muscle phosphofructokinase, the rate-limiting enzyme in glycolysis. These findings suggest that Li^+ causes a reduction in glycolysis, and thus may account for the reported decrease of lactate formation in muscle [11].

The decrease in the regulatory activaties of phosphofructokinase and phosphoglucomutase induced by Li^+, results most probably from the Li^+-induced decrease in the level of Glc-1,6-P_2, the activator of both enzymes, for the following reasons: (a) Only the regulatory activity of these enzymes (which is sensitive to activation by Glc-1,6-P_2) was reduced, with no change in their maximal activities. (b) When the activities of both enzymes were assayed in the presence of externally added Glc-1,6-P_2, the effect of Li^+ was abolished. (c) The enzymes from brain and muscles of the Li^+-treated rats were more sensitive to activation by Glc-1,6-P_2 as compared to untreated (control) animals, most probably as a result of the lower tissue content of Glc-1,6-P_2 in the Li^+-treated animals. (d) Lithium exerted a reduction in phosphofructokinase and phosphoglucomutase activities only in those tissues where it also induced a decrease in Glc-1,6-P_2, namely, in the different kinds of muscles and in brain. In liver, on the other hand, where Li^+ did not affect Glc-1,6-P_2 level, it also did not affect these enzymes' activities. (e) Our previous studies have revealed that many other conditions or treatments which affect Glc-1,6-P_2 concentration, also lead to concomitant changes in the activities of phosphofructokinase and phosphoglucomutase, and it is unlikely to assume that such correlation may be incidental. For example, under all other conditions where we found a decrease in Glc-1,6-P_2, such as in muscular dystrophy [25, 26], treatment with phospholipase A [20], Ca^{2+}-ionophore A23187 [27], or local anesthetics [28], this decrease was accompanied by a concomitant reduction in the activities of phosphofructokinase and phosphoglucomutase. And, vice versa, conditions which lead to an increase in Glc-1,6-P_2, such as growth [19], epinephrine or dibutyryl cyclic AMP [18], result in an activation of phosphofructokinase [18, 23] and phosphoglucomutase [18, 19].

In the dystrophic muscle, where Glc-1,6-P_2 is markedly reduced [25, 26], we found that Li^+ caused an additional decrease in the level of this regulator [29]. Based on the present findings which show that Li^+ exerts a marked reduction in phosphofructokinase activity in muscle, we may assume that Li^+ treatment of patients afflicted with muscular dystrophy, may result in a more severe depression of the already depressed phosphofructokinase activity and glycolysis in the dystrophic muscle [25], and thus may aggravate the muscle damage in this disease.

The markedly elevated urinary cyclic AMP levels in the manic state were reported to be decreased when normal behavior returns after Li^+ therapy [30]. It has been reported by several investigators that Li^+ inhibits hormone-induced activation of adenyl cyclase in different tissues [30, 31]. Previous studies from this laboratory [32] have revealed that dibutyryl cyclic AMP and dibutyryl cyclic GMP act in a "Yin–Yang" manner [33, 34], exerting opposite effects on the levels of Glc-1,6-P_2 and on the activity of phosphofructokinase in the diaphragm muscle: cyclic AMP increased, whereas cyclic GMP decreases these parameters. It is possible that the effects of Li^+ shown in the present paper may be related to changes in the cyclic nucleotides ratio. It should be pointed out that Li^+ exerts opposite effects on glucose metabolism to those exerted by epinephrine or cyclic AMP. Epinephrine, or cyclic AMP, which decrease glycogen content and increase lactate formation in muscle [35], were shown to increase Glc-1,6-P_2 level [18] and to activate phosphoglucomutase [18] and phosphofructokinase [18, 23]. On the other hand, we show here that Li^+, which was reported to increase glycogen content and decrease lactate formation [11], causes a reduction in the concentration of Glc-1,6-P_2 and in the activities of phosphoglucomutase and phosphofructokinase in muscles and brain. It is possible that the reduction in phosphofructokinase and thus in glycolysis induced by lithium, may play an important role in the mechanism of the therapeutic action of this agent in the manic state.

Acknowledgements—This research was carried out under grants from the Muscular Dystrophy Association of America and the Ministry of Industry, Commerce and Tourism, Office of the Chief Scientist, Jerusalem, Israel. Mrs. H. Morgenstern is acknowledged for her invaluable technical assistance.

REFERENCES

1. R. Beitner, *Trends Biochem. Sci.* **4**, 228 (1979).
2. H. W. Hofer and D. Pette, *Hoppe-Seyler's Z. Physiol. Chem.* **349**, 1378 (1968).
3. J. Krzanowski and F. M. Matschinsky, *Biochem. biophys. Res. Commun.* **34**, 816 (1969).
4. R. Beitner, S. Haberman and T. Cycowitz, *Biochim. biophys. Acta* **482**, 330 (1977).
5. S. Rapoport, in *The Human Red Cell* in Vitro (Eds. T. S. Greenwalt and G. A. Jamieson) p. 153. Grune and Stratton, New York (1974).
6. I. A. Rose and J. V. B. Warms, *Biochem. biophys. Res. Commun.* **59**, 1333 (1974).
7. L. F. Leloir, R. E. Trucco, C. E. Cardini, A. C. Paladini and R. Caputto. *Archs Biochem.* **19**, 339 (1948).
8. R. Beitner, S. Haberman and L. Livni, *Biochim. biophys. Acta* **397**, 355 (1975).
9. I. A. Rose and J. B. Warms, *Biochem. biophys. Res. Commun.* **92**, 1030 (1980).
10. F. V. De Feudis, *Archs Int. Pharmacodyn. Ther.* **197**, 141 (1972).
11. T. Clausen, *Biochim. biophys. Acta* **150**, 66 (1968).
12. G. Bhattacharya, *Biochim. biophys. Acta* **93**, 644 (1964).
13. R. A. Mickel, L. Hallidy, N. Haugaard and E. S. Haugaard, *Biochem. Pharmac.* **27**, 799 (1978).
14. G. M. Dempsey, D. L. Dunner, R. R. Fieve, T. Farkas and J. Wong. *Am. J. Psychiat.* **133**, 9 (1976).
15. E. S. Haugaard, A. Frazer, J. Mendels and N. Haugaard, *Biochem. Pharmac.* **24**, 1187 (1975).
16. R. Beitner and N. Kalant, *J. biol. Chem.* **246**, 500 (1971).
17. J. V. Passonneau, O. H. Lowry, D. W. Schulz and J. G. Brown, *J. biol. Chem.* **244**, 902 (1969).
18. R. Beitner, S. Haberman and J. Nordenberg, *Molec. cell. Endocr.* **10**, 135 (1978).

19. R. Beitner, J. Nordenberg and T. J. Cohen, *Int. J. Biochem.* **10**, 603 (1979).
20. R. Beitner, J. Nordenberg, T. J. Cohen and E. Beery, *Int. J. Biochem.* **11**, 467 (1980).
21. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
22. T. E. Mansour, *J. biol. Chem.* **238**, 2285 (1963).
23. T. E. Mansour, *J. biol. Chem.* **247**, 6059 (1972).
24. T. E. Mansour, in *Current Topics in Cellular Regulation* (Eds. B. L. Horecker and E. R. Stadtman), Vol. 5, p. 1. Academic Press, New York (1972).
25. R. Beitner, S. Haberman, J. Nordenberg and T. J. Cohen, *Biochim. biophys. Acta* **542**, 537 (1978).
26. R. Beitner and J. Nordenberg, *FEBS Lett.* **98**, 199 (1979).
27. E. Beery, S. Klein, J. Nordenberg and R. Beitner, *Biochem. Internat.* **1**, 526 (1980).
28. J. Nordenberg, S. Klein, E. Beery, M. Kaplansky and R. Beitner, *Int. J. Biochem.* **13**, 1005 (1981).
29. J. Nordenberg, M. Kaplansky and R. Beitner, *IRCS Med. Sci.* **8**, 880 (1980).
30. I. Singer and D. Rotenberg, *N. Engl. J. Med.* **289**, 254 (1973).
31. P. Thams and A. Geisler, *Acta Pharmac. Tox.* **45**, 329 (1979).
32. R. Beitner and T. J. Cohen, *FEBS Lett.* **115**, 197 (1980).
33. N. D. Goldberg, M. K. Haddox, S. E. Nicol, D. B. Glass, C. H. Sanford, F. A. Kuehl, Jr. and R. Estensen, in *Advances in Cyclic Nucleotide Research* (Eds. G. I. Drummond and P. Greengaard), Vol. 5, p. 307. Raven, New York (1975).
34. N. D. Goldberg and M. K. Haddox, *A. Rev. Biochem.* **46**, 823 (1977).
35. A. Bevitz, E. Mohme-Lundholm and N. Svedmyr, *Acta Physiol. Scand.* **69**, 213 (1967).