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Lithium Inhibits Hepatic Gluconeogenesis and Phosphoenolpyruvate Carboxykinase Gene Expression*

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Fatima Bosch‡, Joan Enric Rodriguez-Gil§, Maria Hatzoglou¶, Anna M. Gomez-Foix‖, and Richard W. Hanson¶

From the Department of Biochemistry and Molecular Biology, School of Veterinary Medicine, Autonomous University of Barcelona, 08193-Bellaterra, Barcelona, Spain and the ¶Pew Center for Molecular Nutrition and the Department of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

Incubation of isolated hepatocytes from fasted rats with 20 mM LiCl for 1 h decreased glucose production from lactate, pyruvate, and alanine. In addition, phosphoenolpyruvate carboxykinase (PEPCK) gene expression in FTO-2B rat hepatoma cells was inhibited by treatment with LiCl. Lithium was also able to counteract the increased PEPCK mRNA levels caused by both Bt_2cAMP and dexamethasone, in a concentration-dependent manner. A chimeric gene containing the PEPCK promoter (−550 to +73) linked to the amino-3-glycosyl phosphotransferase (neo) structural gene was transduced into FTO-2B cells using a Moloney murine leukemia virus-based retrovirus. In these infected cells, 20 mM LiCl decreased both the concentration of neo mRNA transcribed from the PEPCK-neo chimeric gene and mRNA from the endogenous PEPCK gene. Lithium also inhibited the stimulatory effect of Bt_2cAMP and dexamethasone on both genes. The stability of neo mRNA was not altered by lithium, since in cells infected with retrovirus containing only the neo gene transcribed via the retroviral 5′-LTR and treated with 20 mM LiCl, no change in neo mRNA levels was observed. The intraperitoneal administration of LiCl to rats caused a decrease in hepatic PEPCK mRNA, indicating that lithium could also modify gene expression *in vivo*. The effects of lithium were not due to an increase in the concentration of insulin in the blood but were correlated with an increase in hepatic glycogen and fructose 2,6-bisphosphate levels. These results indicate that lithium ions, at concentrations normally used therapeutically for depression in humans, can inhibit glucose synthesis in the liver by a mechanism which can selectively modify the expression of hepatic phosphoenolpyruvate carboxykinase.

Lithium is known to influence carbohydrate metabolism. It can stimulate glucose transport and utilization and increase glycogen synthesis in isolated hepatocytes (1–3), adipocytes (4, 5), and diaphragm (6, 7). For this reason, lithium has been considered an insulin-like agent. On the other hand, lithium also has a profound effect on calcium mobilizing hormones, by reducing the supply of inositol required to maintain the membrane inositol lipids used to generate the second messengers inositol 1,4,5-triphosphate and diacylglycerol (8–10). However, little information is available regarding the effects of lithium on the regulation of gene expression. It has been reported that lithium can potentiate the effects of the combination of nerve growth factor, dexamethasone, and forskolin on the levels of neurotensin/neuromedin mRNA (11). No direct effect of lithium on gene expression has so far been described.

Since previous reports showed that lithium regulated glycogen metabolism in hepatocytes (1, 3), we investigated whether it might modify hepatic glucose metabolism and affect the expression of the gene for phospho*enol*pyruvate carboxykinase (GTP) (EC 4.1.1.1.32) (PEPCK),[1] a key enzyme in gluconeogenesis (12). The expression of the PEPCK gene is increased by Bt_2cAMP (13–17), dexamethasone (15–17), and inhibited by insulin (18–20), the phorbol ester, TPA (21), and vanadate (22). Different hormone regulatory elements which bind nuclear proteins have already been identified in the 5′-flanking promoter/regulatory region (−550 to +73) of the gene (14–17, 19, 23). Several of these regulatory elements have also been shown to bind nuclear proteins (24, 25) which may be involved in the control of PEPCK gene transcription.

We find that lithium ions inhibit PEPCK gene expression in rat hepatoma cells. In addition, acute administration of lithium to rats alters hepatic glucose metabolism and PEPCK gene expression, indicating that lithium has an antigluconeogenic effect both *in vitro* and *in vivo*.

EXPERIMENTAL PROCEDURES

Materials—The random primer labeling kit was obtained from Boehringer Mannheim. [α-^{32}P]dCTP (3000 Ci/mmol) and GeneScreen Plus were purchased from Du Pont-New England Nuclear. All media, sera, and G418 were from Gibco Laboratories. Lithium chloride was from Fisher or Merck. Bt_2cAMP and dexamethasone were from Sigma. The other reagents used in this study were of the highest purity available.

Thymidine kinase-deficient rat hepatoma (FTO-2B) cells (provided by Dr. Keith Fournier, Fred Hutchison Cancer Center, Seattle, WA) are a well differentiated hepatoma cell line, with hepatocyte morphology, which express liver-specific proteins, such as PEPCK and albumin (14, 26).

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‡ Recipient of a Fogarty International Research Fellowship. To whom correspondence and reprint requests should be addressed.

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[1] The abbreviations used are: PEPCK, phosphoenolpyruvate carboxykinase; kb, kilobase(s); EGTA, [ethylenebis(oxyethylenenitrilo)] tetraacetic acid; CAT, chloramphenicol acetyltransferase; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

Preparation and Incubation of Hepatocytes—Suspensions of isolated parenchymal liver cells were obtained from the livers of 48-h starved, male Sprague-Dawley rats (180–250 g body weight) (27). Hepatocytes were resuspended in Krebs bicarbonate buffer (pH 7.4), free of glucose. Aliquots (3.5 ml, 4–5 × 10^6 cells/ml) were incubated at 37 °C with continuous shaking. The cells were preincubated for 30 min with 20 mM lactate, pyruvate, or alanine, before the addition of 20 mM LiCl.

Infection of the FTO-2B Hepatoma Cells with Recombinant Retrovirus—The construction and the characteristics of the recombinant retroviral plasmid pLJPCK, as well as the procedures for DNA transfection of Ψ 2 cells, viral production, and the infection of FTO-2B cells, have been described in detail by Hatzoglou *et al.* (23) and Bosch *et al.* (22).

DNA Probes—The following probes have been used for DNA/RNA hybridization experiments: PEPCK cDNA, 1.1-kb *Pst*I-*Pst*I fragment from the 3′-end of the PEPCK cDNA, pPCK10 (28); neo, 1-kb *Bgl*II-*Eco*RI fragment from the 5′-end of the neo gene (23). All DNA probes were labeled using [α-^{32}P]dCTP, by the method of random oligopriming as described by the manufacturer. The specific activity of the DNA probe labeled in this manner was 10^9 cpm/μg of DNA.

Isolation and Analysis of RNA from Cells and Liver—Isolation of total RNA was carried out by standard procedures which have been described in detail previously (14). Northern blotting analysis and agarose gel electrophoresis were performed by standard methods (29, 30).

Hormonal Treatment of FTO-2B Cells—FTO-2B cells were placed in serum-free Dulbecco's minimal essential medium 18 h prior to any treatment. Cells were then incubated with either 0.5 mM Bt_2cAMP plus 1 mM theophylline or 1 μM dexamethasone. The concentration of LiCl used in individual experiments is indicated under "Results."

Lithium Treatment in Vivo—The effects of LiCl were studied in fed or 24-h starved Sprague-Dawley rats (180–250 g body weight), which had been injected intraperitoneally with LiCl (85 mg of LiCl/kg body weight). A second injection of the same dose was administered to the rats 90 min later. Finally, 90 min after the last injection, the animals were killed by decapitation. Fragments of liver were frozen, and blood was collected to measure lithium, glucose, and insulin levels.

Enzyme and Metabolite Assays—Glucose was measured in serum and in the 12000 × *g* supernatants of hepatocyte suspensions by the hexokinase method (GlucoQuant System, Boehringer Mannheim). The levels of lithium in the serum were determined in diluted samples by flame spectrophotometry (Instrumentation Laboratory 943, Automatic System), with cesium standards. The concentration of insulin in the blood was determined by radioimmunoassay (System INSIK-5, Soren Biomedica). The activity ratio of hepatic glycogen synthase and the activity of glycogen phosphorylase a was measured in 10,000 × *g* supernatants of liver homogenates. The homogenization buffer contained 10 mM Tris-HCl (pH 7.4) with 150 mM potassium fluoride, 15 mM EDTA, 0.6 M sucrose, 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 25 μg/ml leupeptin, and 50 mM β-mercaptoethanol. The glycogen synthase activity ratio was measured using the low glucose-6-P/high glucose-6-P method as described by Guinovart *et al.* (31). Glycogen phosphorylase a activity was determined as described by Van der Werve *et al.* (32). The protein concentration was determined by the biuret method (33), as described by Layne (34). To measure 6-phosphofructo-2-kinase a activity, liver samples were homogenized in ice-cold buffer (pH 7.0) containing 20 mM potassium phosphate, 10 mM EDTA, and 100 mM KCl. To determine pyruvate kinase a activity, liver was homogenized in a buffer containing 100 mM potassium fluoride, 15 mM EGTA, and 50 mM glycylglycine (pH 7.4). Both activities were analyzed in 12,000 × *g* supernatants as described in Refs. 35 and 36, respectively.

The concentrations of hepatic glycogen and glucose 6-phosphate were measured in perchloric extracts, which were adjusted to pH 5 with 5 M K_2CO_3 to determine glycogen and to pH 7 for glucose 6-phosphate. Glycogen levels were determined by using the α-amyloglucosidase method (37), while glucose 6-phosphate was measured enzymatically (38). Liver fructose 2,6-bisphosphate content was measured in liver samples homogenized in 54 mM NaOH, heated at 80 °C, and then centrifuged at 12,000 × *g* for 10 min. The fructose 2,6-bisphosphate levels of supernatants were determined by its ability to activate PP_i:fructose-6-phosphate-1-phosphotransferase, by the method described by Van Schaftingen *et al.* (39).

RESULTS

Effects of Lithium on the Rate of Gluconeogenesis by Isolated Hepatocytes—Hepatocytes isolated from 48-h starved rats were incubated in Krebs-Henseleit buffer containing: 20 mM lactate, 20 mM pyruvate, or 20 mM alanine, with or without 20 mM LiCl. At 30-min intervals, an aliquot of the cell suspension was removed from the vial, and glucose production was measured. Lithium inhibited (about 25%) glucose production from the three gluconeogenic precursors tested (Fig. 1). These results indicate that lithium ions are able to inhibit gluconeogenesis by isolated hepatocytes.

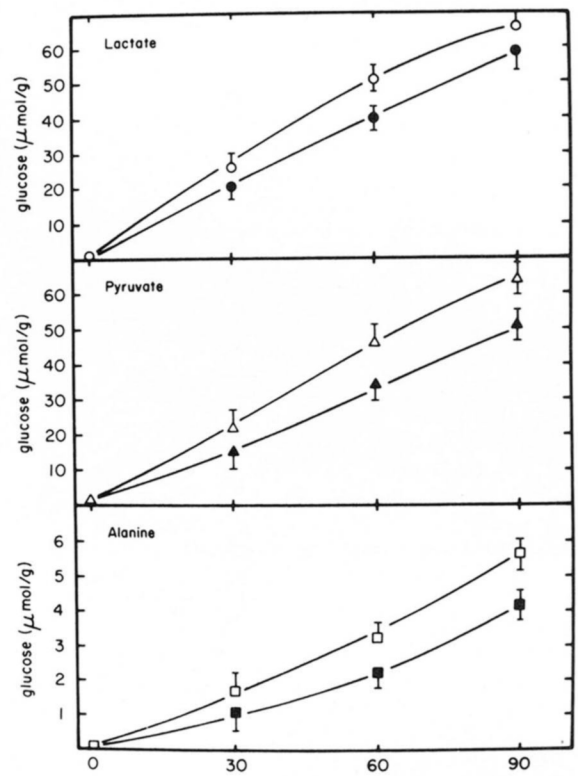

FIG. 1. **Lithium effects on glucose production from gluconeogenic precursors in isolated hepatocytes.** Hepatocytes from starved rats were incubated with 20 mM lactate, 20 mM pyruvate, or 20 mM alanine in the absence of (○, △, □) or presence (●, ▲, ●) of 20 mM LiCl. The results are the means ± S.E. for four different experiments. The horizontal axis represents time in minutes.

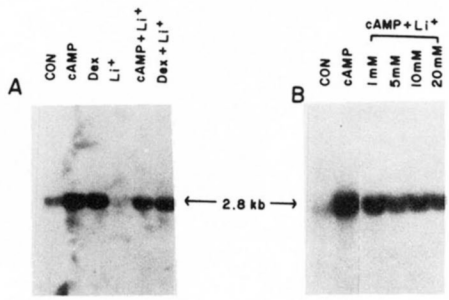

FIG. 2. **Effects of lithium on PEPCK gene expression in FTO-2B rat hepatoma cells.** *A*, the level of RNA for endogenous PEPCK was determined by Northern analysis of RNA isolated from cells which were treated for 4 h with medium (no hormones added) (*CON*), 20 mM LiCl (*Li*$^+$), 0.5 mM Bt_2cAMP, and 1 mM theophylline (*cAMP*), or 1 μM dexamethasone (*Dex*). *B*, FTO-2B cells were treated with 0.5 mM Bt_2cAMP and 1 mM theophylline or with Bt_2cAMP plus the indicated concentrations of LiCl for 4 h. RNA was extracted from the cells, and the concentration of PEPCK mRNA was determined by Northern blotting, using PEPCK cDNA as a hybridization probe.

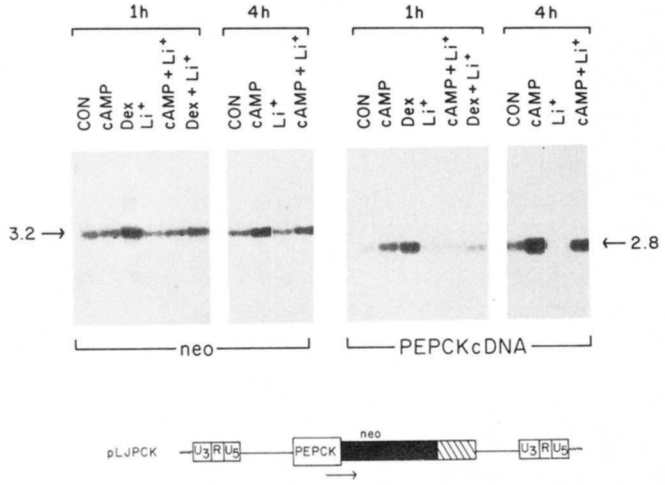

FIG. 3. **Effects of lithium on FTO-2B cells infected with vLJPCK.** FTO-2B cells were infected with vLJPCK and maintained in culture with G418. The levels of neo and endogenous PEPCK RNA were determined by Northern analysis using RNA isolated from cells treated for 1 h or 4 h with 0.5 mM Bt_2cAMP and with 1 mM theophylline or 1 μM dexamethasone, with or without 20 mM LiCl. The vector used for the infection of the FTO-2B cells is shown at the *bottom* of the figure.

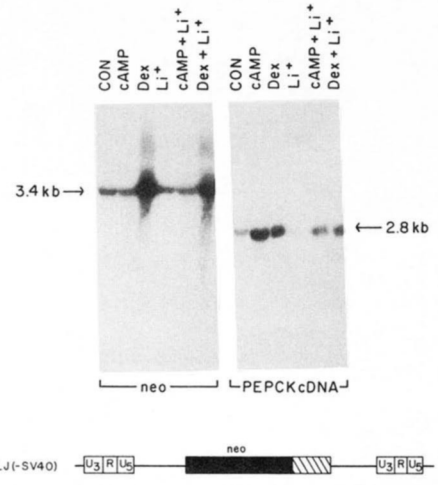

FIG. 4. **Effects of lithium on RNA from FTO-2B cells infected with vLJ(−SV40).** FTO-2B cells were infected with vLJ(−SV40) and grown in medium containing G418 for 2 months. The level of RNA for the neo and endogenous PEPCK genes was determined by Northern analysis of RNA isolated from these cells after treatment for 4 h with 0.5 mM Bt_2cAMP and 1 mM theophylline or 1 μM dexamethasone in the presence or absence of 20 mM LiCl. The 3.4-kb RNA transcript initiates within the 5′-LTR, whereas the 2.8-kb band represents the mRNA from the endogenous PEPCK gene. The vector used for the infection of the FTO-2B cells is shown at the *bottom* of the figure.

Effects of Lithium Ions on PEPCK Gene Expression—Treatment of FTO-2B hepatoma cells with 20 mM LiCl for 4 h decreased the levels of PEPCK mRNA (Fig. 2*A*). Incubation of the cells with Bt_2cAMP plus theophylline or with dexamethasone increased PEPCK gene expression. LiCl, added together with cyclic nucleotide or dexamethasone, blocked the effects of these compounds on the induction of PEPCK mRNA (Fig. 2*A*). The effect of LiCl was concentration-dependent; 1 mM LiCl caused a 50% reduction in the normally observed induction of PEPCK mRNA by Bt_2cAMP (Fig. 2).

NaCl treatment of the cells did not produce an inhibition of PEPCK gene expression (data not shown), indicating that the effects of LiCl were not the result of an increase in the salt content of the incubation media. No effect of LiCl on actin mRNA levels has been observed (data not shown), and hybridization with actin cDNA has been used to normalize mRNA on the blots.

TABLE I

The concentration of lithium, insulin, and glucose in the blood of LiCl-treated rats

Lithium, insulin, and glucose were measured, as described under "Experimental Procedures," in eight rats from each experimental group. Results are mean ± S.E. Statistical significance was determined by paired Student's *t* test.

	Lithium	Insulin	Glucose
	mM	*ng/ml*	*mM*
24-h starved rats			
Control	0.02 ± 0.02	0.85 ± 0.13	4.9 ± 0.3
Lithium-treated	1.73 ± 0.15[a]	0.54 ± 0.08[a]	4.5 ± 0.3
Fed rats			
Control	0.03 ± 0.04	24.2 ± 3.8[b]	8.0 ± 0.9[b]
Lithium-treated	1.68 ± 0.19[a]	16.4 ± 1.0[a]	7.6 ± 0.7

[a] $p \leq 0.01$ control *versus* lithium-treated rats.
[b] $p \leq 0.01$ control starved *versus* control fed rats.

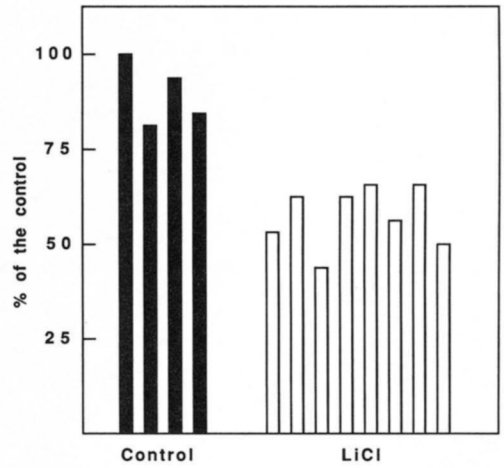

FIG. 5. **Effects of lithium on hepatic PEPCK gene expression.** The level of RNA for PEPCK was determined by Northern analysis of RNA isolated from liver of rats acutely treated with LiCl as described under "Experimental Procedures." The data are expressed as -fold induction over the highest control. Figure shows the results corresponding to 4 control (*closed bars*) and 8 lithium-treated (*open bars*) rats.

In order to determine if lithium inhibited PEPCK gene transcription, FTO-2B cells were infected with replication-incompetent virus containing the PEPCK promoter (−550 to +73) ligated to the structural neo gene, and the effect of lithium on neo mRNA was measured by Northern blotting. Treatment of the cells for 1 h with dexamethasone or 4 h with Bt_2cAMP plus theophylline induced the 3.2-kb neo RNA transcript, which is produced when the transcription is initiated at the PEPCK promoter and the newly synthesized RNA is polyadenylated within the 3′-LTR (Fig. 3). LiCl (20 mM) produced a decrease in the expression of the chimeric PEPCKneo gene, and, when added together with dexamethasone or Bt_2cAMP, also blocked the induction of neo gene expression by both effectors (Fig. 3). The effect of LiCl on the expression of the endogenous PEPCK gene present in FTO-2B cells infected with vLJPCK was also measured on the same Northern blot. LiCl, alone and in the presence of dexamethasone or Bt_2cAMP, decreased the concentration of PEPCK mRNA, either after 1 or 4 h of the treatment of the cells (Fig. 3).

We next studied the effects of LiCl on the expression of

TABLE II

Effects of lithium treatment on the metabolism of hepatic glycogen

Glycogen and glucose 6-phosphate levels, the glycogen synthase activity ratio, and the glycogen phosphorylase activity were determined as described under "Experimental Procedures." There were 8 rats in each experimental group. The results are expressed as the mean ± S.E., and statistical significance was obtained using the Student's *t* test.

	Glycogen synthase activity ratio	Glycogen phosphorylase *a*	Glycogen	Glucose-6-P
		milliunits/mg protein	*mg/g liver*	*μmol/g liver*
24-h starved rats				
Control	0.37 ± 0.04	80.1 ± 4.0	1.0 ± 0.2	0.08 ± 0.01
Lithium-treated	0.59 ± 0.07[a]	79.0 ± 4.5	2.2 ± 0.5[a]	0.18 ± 0.05[a]
Fed rats				
Control	0.44 ± 0.07	121.5 ± 9.1[b]	36.6 ± 8.7[b]	0.40 ± 0.07[b]
Lithium-treated	0.66 ± 0.05[a]	114.3 ± 5.0	66.8 ± 13.9[a]	0.64 ± 0.05[a]

[a] $p \leq 0.01$ control *versus* lithium-treated rats.
[b] $p \leq 0.01$ control starved *versus* control fed rats.

TABLE III

Effects of lithium treatment on the levels of fructose 2,6-bisphosphate levels and on 6-phosphofructo-2-kinase and pyruvate kinase in rat liver

Parameters were measured as described under "Experimental Procedures." Results are means ± S.E. of 8 rats from each experimental group. Statistical differences were determined by using the Student's *t* test.

	Fructose 2,6-bisphosphate	6-phosphofructo-2-kinase *a*	Pyruvate kinase *a*
	nmol/g liver	*nmol/min × g liver*	*milliunits/mg liver*
24-h starved rats			
Control	0.27 ± 0.03	1.0 ± 0.3	14.0 ± 0.4
Lithium-treated	0.37 ± 0.04[a]	1.9 ± 0.2[b]	14.9 ± 0.7
Fed rats			
Control	13.07 ± 3.13[c]	7.1 ± 1.3[c]	25.1 ± 1.5[c]
Lithium-treated	21.09 ± 2.55[a]	11.4 ± 0.5[a]	31.3 ± 2.9[a]

[a] $p \leq 0.01$ control *versus* lithium-treated rats.
[b] $p \leq 0.05$ control *versus* lithium-treated rats.
[c] $p \leq 0.01$ control starved *versus* control fed rats.

neo gene in FTO-2B-infected cells with the vLJ(−SV40). This provirus has no internal promoter linked to the neo gene, with transcription initiation at the 5′-LTR. Northern blot was hybridized with either a neo or a PEPCK cDNA probe. The neo probe hybridized with a 3.4-kb RNA which correspond to the full length viral transcript containing neo (Fig. 4). Treatment of the cells with LiCl or Bt_2cAMP did not significantly alter neo gene transcription. In contrast, the addition of dexamethasone markedly increased the neo mRNA levels, indicating that this hormone can control the expression of a structural gene included in the provirus by altering transcription from the 5′-LTR, probably due to the presence of a glucocorticoid regulatory element in the LTR. LiCl was unable to modify this effect of dexamethasone, indicating that the stability of neo mRNA is not altered by lithium.

The same Northern blot, hybridized with the PEPCK cDNA, showed that LiCl blocked both the basal and Bt_2cAMP or dexamethasone induction of endogenous PEPCK mRNA (Fig. 4).

Acute Effects of LiCl on PEPCK Gene Expression in the Liver of Starved Rats—Rats starved for 24 h were injected intraperitoneally with LiCl (85 mg of LiCl/kg of body weight), and the concentration of lithium in the blood was determined (Table I). We also determined blood insulin levels in control and lithium-treated animals (Table I). Northern blotting analysis showed that rats treated with LiCl had lower levels of PEPCK mRNA than control animals (Fig. 5). This effect was caused by lithium since insulin levels were lower in lithium-treated rats than in controls.

Acute Effects of Lithium on Hepatic Glucose Metabolism—Since lithium, after acute administration to animals, was able to modify PEPCK gene expression, we investigated if these effects were related to changes in hepatic glucose metabolism in fed and 24-h starved rats. Table I shows that in fed animals lithium treatment also provoked a decrease in the level of insulin in the blood. However, lithium did not modify the concentration of blood glucose in either fed or fasted animals (Table I). Lithium treatment caused a significant increase in the concentration of hepatic glycogen in both fed and starved rats (Table II). This effect was correlated with an increase in the glycogen synthase activity ratio and with the glucose 6-phosphate levels. The activities of glycogen phosphorylase a were not altered by this treatment (Table II). Lithium also increased the concentration of hepatic fructose 2,6-bisphosphate and the activity of 6-phosphofructo-2-kinase a activity, in both starved and fed rats (Table III). Pyruvate kinase a activity was slightly increased in fed animals (Table III). These effects correlate well with the decrease of PEPCK gene expression observed in these animals, indicating that acutely administered lithium induced glucose utilization.

DISCUSSION

In the present work, we show that lithium treatment of FTO-2B hepatoma cells resulted in a decrease in the level of PEPCK mRNA. In addition, acute lithium treatment of rats also decreases the expression of the gene for PEPCK. These are the first observations of a direct effect of lithium on gene transcription. Moreover, this cation was able to counteract the increased mRNA levels caused by Bt_2cAMP or dexamethasone, in noninfected FTO-2B cells or in cells infected with vLJPCK. This counteracting effect of lithium on PEPCK-gene expression was similar to that exerted by insulin (18–20) or phorbol esters such as TPA (21). A previous report (11) showed that, in PC12 pheochromocytoma cells, lithium potentiated the increase in mRNA levels of the neuropeptide neurotensin caused by a combination of nerve growth factor, dexamethasone, and the adenylate cyclase activator, forskolin. These effects were surprising since lithium in other systems inhibits cellular response involving cAMP (40, 41), in agreement with our results. However, lithium by itself was unable to induce the expression of neurotensin gene (11), indicating that in this system it might modulate the action of other effectors. In this regard, it has also been shown that lithium potentiates tumor necrosis factor-mediated cytotoxicity in several tumor cell lines and in nude mice injected with

L929 tumor cells, whereas little effect was observed when TNF or lithium was administered alone (42).

The effects of lithium on PEPCK mRNA in hepatoma cells were dependent on the concentration of the LiCl used. Even though 1 mM LiCl was already effective, stronger effects appeared with higher concentrations. It is worth nothing that therapeutic levels of lithium in the blood of humans is about 1 mM. At this concentration, we note effects on glucose and glycogen metabolism and on PEPCK gene expression in rats.

The reduction in the levels of PEPCK mRNA caused by LiCl was detected after 1 h of cell incubation. This rapid response to lithium suggests that this compound did not act by stabilizing PEPCK mRNA. Evidence indicating that lithium acted on the transcription of PEPCK gene was obtained using FTO-2B cells infected with vLJPCK. This retrovirus contained a chimeric gene formed by the fusion of the 550 base pairs of the PEPCK promoter, which include regulatory elements, to the neo structural gene (23). It has been demonstrated that when this chimeric gene was introduced into hepatoma cells, transcription from the PEPCK promoter was predominant over the 5'-LTR of retrovirus, and the level of transcription could be regulated by hormones (12, 23). Treatment of these infected cells with lithium caused a decrease in both the level of transcription from the PEPCK promoter in the retrovirus and the concentration of PEPCK from the endogenous PEPCK gene.

Changes in PEPCK gene expression could be related to modifications in hepatic glucose metabolism. In this regard, hepatocytes treated with lithium showed a decrease in gluconeogenesis from different precursors. Similar results were obtained in acute lithium-treated rats. In these animals, a decrease in PEPCK gene expression correlated with an increase in fructose 2,6-bisphosphate, an important regulator of the fructose 6-phosphate/fructose 1,6-bisphosphate cycle in liver (43). An increase in fructose 2,6-bisphosphate results in a stimulation of the rate of glycolysis and a decrease on gluconeogenesis (43). We also found an induction of 6-phosphofructo-2-kinase activity in lithium-treated rats. This effect could be the result of an increase in the expression of the gene for this enzyme. Studies performed using isolated hepatocytes showed that after a short treatment (up to 60 min) with lithium, no modification of 6-phosphofructo-2-kinase activity was detected. On the other hand, chronic lithium treatment of rats also provoked changes on 6-phosphofructo-2-kinase activity that could be related to modifications in expression of its gene.[2] Lithium also caused an increase in the glycogen content, which correlated with a raise in the concentration of hepatic glucose 6-phosphate and glycogen synthase activity. Similar effects have been described in isolated hepatocytes (1–3), adipocytes (4, 5), and diaphragm (6, 7) or in muscle of chronic lithium-treated animals (44). This insulin-like effect of acute lithium treatment on liver of rats was not mediated by an increase in insulin in the blood, since the concentration of insulin in both starved and fed rats was decreased by lithium. It has been reported that lithium can inhibit glucose-stimulated insulin secretion either *in vivo* (45) or in isolated pancreatic islets (46). Our results indicate that lithium can also directly affect hepatic metabolism.

The mechanism(s) by which lithium exerts its effects on cellular processes are not clear. While it is well known that lithium acts as an uncompetitive inhibitor of inositol phosphate metabolism (8–10), it can also block adrenergically activated adenylate cyclase (47–49) and perturb G-protein receptor coupling (50). Although these actions explain the antidepressant effects of lithium, there are no data relating these mechanisms with the effects of lithium on glucose metabolism. In this regard, lithium activates hepatocyte glycogen synthase even in the absence of calcium in the incubation medium (3). However, since lithium can exert a profound effect on early embryonic development by an inositol-dependent mechanism (10), it is possible that a similar type of mechanism is also involved in the action of lithium on PEPCK gene expression.

A major question arising from this study concerns the mechanism by which lithium exerts its effects on PEPCK gene transcription. Transcription from the PEPCK promoter is regulated by a number of interacting elements which are present in the segment of the promoter (−550 to +73) used in this study. Two regulatory elements, CRE-1 (−74 to −87) and P3(I) (−230 to −248) are specially important in the cAMP responsiveness of the PEPCK promoter. The cAMP regulatory element binding protein (CREB) binds at CRE-1 and both CAAT/enhancer binding protein (C/EBP) and liver activating protein (LAP) bind to P3(I) (51). Co-transfection of the genes for these transcription factors together with a chimeric PEPCK/CAT gene stimulated transcription from the PEPCK promoter, while substitution mutations in these two regions of the promoter completely blocked both basal and cAMP-stimulated transcription (51). Immediately adjacent to P3(I) is a thyroid hormone regulatory element which functions synergistically with P3(I) and CRE-1 to enhance the level of PEPCK gene transcription. A region from −380 to −420 has also been implicated in the glucocorticoid regulation of PEPCK gene transcription (52). This area contains a glucocorticoid regulatory element as well as two accessory protein binding domains which are required for the full stimulation of PEPCK gene transcription by glucocorticoids (53). Finally, Jun has been shown to stimulate transcription from the PEPCK promoter in hepatoma cells; an effect which can be reversed by co-transfection of an expression vector containing the gene for c-*fos*.[3]

The regions of the PEPCK promoter involved in the negative control of gene transcription are less well understood. Insulin (54) and vanadate (22) decrease both the basal rate of PEPCK gene transcription and block the positive effect of cAMP and glucocorticoids on transcription. Recently, we have shown that protein kinase C, when introduced into hepatoma cells by transfection of an expression vector, inhibits transcription from the PEPCK promoter and also blocks the stimulatory effects of both the catalytic subunit of protein kinase A and cAMP.[4] Protein kinase C exerts its effects at CRE-1 and interferes with the binding and/or stimulation of transcription from the PEPCK promoter normally associated with CREB.

Lithium causes a large accumulation of diacylglycerol (55) which is known to activate protein kinase C. It is possible that lithium exerts its effects on PEPCK gene transcription by a cascade involving an elevation in the concentration of diacylglycerol in the liver, followed by an activation of protein kinase C, which in turn inhibits transcription. In preliminary studies using the PEPCK promoter deleted to −109, we noted that cAMP-stimulated transcription was blocked by the addition of lithium to hepatoma cells.[2] This suggests that lithium exerts its effects at CRE-1, the same region which is responsive to protein kinase C. However, a direct link between lithium and protein kinase C in their effects on PEPCK gene transcription remains to be established.

[2] F. Bosch, unpublished observations.

[3] A. J. Gurney, unpublished observations.

[4] E. A. Park, unpublished observations.

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