Stimulatory effect of lithium on glucose transport in rat adipocytes is not mediated by elevation of IP$_1$

XIAOLI CHEN, ELLEN G. MCMAHON, AND ERIC A. GULVE
Cardiovascular Disease and Diabetes Research, Monsanto Company, St. Louis, Missouri 63167

Chen, Xiaoli, Ellen G. McMahon, and Eric A. Gulve. Stimulatory effect of lithium on glucose transport in rat adipocytes is not mediated by elevation of IP$_1$. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E272–E277, 1998.—Lithium has been shown to increase glucose uptake in skeletal muscle and adipose tissues. The therapeutic effect of lithium on bipolar disease is thought to be mediated by its inhibitory effect on myo-inositol-1-monophosphatase (IMPase). We tested the hypothesis that the stimulatory effect of lithium on glucose uptake results from inhibition of IMPase and the resultant accumulation of inositol monophosphates (IP$_1$) by comparing the effects of lithium and a selective IMPase inhibitor, L-690,488, on isolated rat adipocytes. Insulin produced a concentration-dependent stimulation of 2-deoxy-D-[${}^{14}$C]glucose (2-DG) transport (10 µU/ml caused half-maximal activation). Acute exposure to lithium stimulated basal glucose transport activity in a concentration-dependent manner, with a threefold stimulation at 30 mM lithium. Lithium also potentiated insulin-stimulated 2-DG transport. Lithium produced a concomitant increase in IP$_1$ accumulation. In contrast, L-690,488 increased IP$_1$ to levels comparable to those of lithium without stimulatory effects on 2-DG transport. These results demonstrate that stimulatory effects of lithium on glucose transport are not mediated by the inhibition of IMPase and subsequent accumulation of IP$_1$ in rat adipocytes.

prodrug: inositol monophosphatase inhibitor; insulin; isolated adipocytes

LITHIUM is a widely used therapeutic agent for the treatment of manic-depressive disorders. The therapeutic effect of lithium on bipolar disorders is believed to result from an inhibitory action on myo-inositol-1-monophosphatase (IMPase) (6). IMPase plays an important role in phosphatidylinositol metabolism in that this enzyme catalyzes the dephosphorylation of inositol monophosphates (IP$_1$) to produce free inositol. Inositol then combines with cytidine monophosphorylphosphatidate to form phosphatidylinositol (17). Thus exposure to lithium could deplete inositol pools in the brain by inhibition of IMPase, leading to reduced cellular responses to neurotransmitters, the receptors of which are coupled to the phosphatidylinositol signal transduction pathway (6).

Lithium has also been shown to affect various endocrine functions (23). For example, lithium enhances glucose transport and metabolism in insulin-sensitive tissues. In manic-depressive disorder patients, lithium treatment improved glucose tolerance (26). A direct effect of lithium on peripheral glucose disposal in vivo has been demonstrated in rats. Lithium was shown to have antidiabetic effects in a pancreatectomized rat model, where lithium at the plasma concentrations attained in the treatment of bipolar disorder completely restored muscle insulin action to normal (22).

METHODS

Preparation of Isolated Adipocytes

Male Sprague-Dawley rats weighing 140–200 g were fed with standard Purina Chow and water ad libitum. Epididymal fat pads were isolated under pentobarbital sodium anesthesia (5 mg/100 g body weight ip), and adipocyte suspensions were prepared by treating the fat pads with a crude collagenase, as described by Rodbell (21) with modifications. In most experiments, epididymal fat pads were isolated from three animals and rinsed in warmed saline solution (pH 7.4).
Each 25-ml polyethylene Erlenmeyer flask contained two or three fat pads that had been pooled and cut into pieces ~2 mm in diameter. All incubations were carried out in a freshly prepared Krebs Ringer phosphate (KRP) buffer (with the following composition in mM: 125 NaCl, 5 KCl, 1 KH₂PO₄, 10 HEPES, 1.25 CaCl₂, 2H₂O, and 1.25 MgSO₄·7H₂O) containing 1% fraction V BSA and at a pH of 7.4 at 37°C. The fat tissues were dispersed into small fragments by incubating for 1 h with 5 ml of collagenase solution (2.5 mM of glucose and 1 mg/ml of collagenase in KRP buffer) in a shaking incubator (120 cycles/min) at 37°C. Cells were then filtered through nylon mesh. All the cells from different flasks were pooled, washed three times with fresh KRP buffer (40 ml/wash), and centrifuged (1,000 rpm for 30 s). The cells were resuspended in KRP buffer to the desired density. An adipocrit of 25% gave a cell suspension of ~1 million adipocytes/ml.

Measurement of Inositol Phosphate Production

Labeling and incubation. These procedures were performed according to the methods of Pennington and Martin (20) with modifications. Isolated adipocytes with an adipocrit of 25–30% (160 mg dry weight/ml) were preincubated in triplicate with myo-[³H]inositol (80 µCi/ml) in a total volume of 750 µl in a shaking water bath for 2 h at 37°C. The cell suspensions were centrifuged and washed four times with KRP buffer in 20× cell volume. To prevent cell loss, during washing and centrifugation, stiff tubing was inserted into the centrifuge tube, and buffer was aspirated through a vacuum line connected to the tubing. The cell suspensions were concentrated to 500 µl per sample after the final wash, and the cells were then incubated with agonists at 37°C for the indicated time periods. The reactions were terminated by transferring 500-µl cell suspensions to 2 ml of ice-cold chloroform-methanol (1:2; vol/vol).

Lipid extraction. Lipids were extracted by the method of Bligh and Dyer (7) with modifications. Thus 0.66 ml of chloroform and 0.66 ml of water were added into the mixture of sample and chloroform-methanol. The phases were separated by centrifugation at 2,500 rpm for 20 min at 4°C. An aliquot of the upper aqueous phase was removed for determination of inositol phosphates (IPs).

Separation and assay of IPs. IPs were measured by the method of Berridge et al. (5). Briefly, the ³H-labeled IPs were separated on anion exchange columns of Dowex 1-X8 (100–200 mesh, formate form, 1 ml). After the aqueous samples were loaded (1.8 ml), the columns were washed with distilled water (50 ml), 5 mM disodium tetraborate, and 60 mM sodium formate (30 ml) to remove free [³H]inositol and [³H]glycerophosphoinositol, respectively. IP₃, inositol trisphosphate (IP₃), and inositol triphosphate (IP₃) were then sequentially eluted with 0.1 M formic acid-0.2 M ammonium formate (30 ml), 0.1 M formic acid-0.4 M ammonium formate (25 ml), and 0.1 M formic acid-0.1 M ammonium formate (25 ml), respectively. These fractions were then counted for radioactivity in a liquid scintillation counter.

Statistical Analysis
For each experiment, data are expressed as means (triplicate or quadruplicate samples for each treatment group) ± SE. The significance of differences in each independent experiment was determined by nonparametric ANOVA and the least squares means procedure for paired groups, or a one-tailed ANOVA followed by Tukey's Studentized range test for multiple comparisons. Experiments were performed multiple times for confirmation (see legends to Figs. 1–5). E₅₀ was estimated from a four-parameter logistic regression equation.

Materials
Rats were purchased from Charles River Laboratory (Portage, MI). Collagenase (type I) was obtained from Worthington Biochemical (Freehold, NJ). Porcine insulin (Iletin II) was purchased from Eli Lilly (Indianapolis, IN). Lithium chloride, cytochalasin B, BSA, and 2-DG were purchased from Sigma Chemical (St. Louis, MO). L-690,488 was obtained from Tocris Cookson (St. Louis, MO). 2-[¹⁴C]DG and [¹⁴C]mannitol were purchased from American Radiolabeled Chemicals (St. Louis, MO). d-myo-[³H]inositol was purchased from Du Pont-NEN (Boston, MA). Dowex 1-X8 was obtained from Bio-Rad Laboratories (Hercules, CA).
RESULTS

Effect of Lithium on Basal Glucose Transport

The basal 2-DG uptake rate was 1.78 nmol·15 min⁻¹·10⁶ adipocytes⁻¹ in the experiment shown in Fig. 1. Lithium increased the basal 2-DG uptake rate in a concentration-dependent manner, with an approximate threefold stimulation at 30 mM. Time-course experiments (2.5–30 min preincubation) demonstrated that lithium (10 mM) stimulated glucose transport activity rapidly, with a maximal effect measured after 5 min of preincubation before the 2-DG transport assay (data not shown). In most of the experiments described below, 30 mM lithium was used to maximize the effects on glucose transport.

Effect of Lithium on Insulin-Stimulated 2-DG Transport

In addition to the effect of lithium on basal 2-DG transport, insulin-induced 2-DG transport was potentiated by lithium (Fig. 2A). The lithium potentiation of insulin action occurred only at submaximal insulin concentrations. The potentiation of lithium on insulin-stimulated 2-DG transport became less marked at insulin concentrations approaching the maximally effective concentration, where the effects of lithium and insulin were merely additive. When 2-DG uptake was expressed as a percentage of the maximal insulin effect, a substantial shift in the insulin concentration-response curve was apparent (Fig. 2B). The effect of lithium to potentiate glucose transport activity at low insulin concentrations was seen reproducibly in several different experiments. These data suggest that, in addition to stimulating glucose transport in the absence of insulin, lithium also enhances insulin sensitivity in rat adipocytes.

Most experiments were performed using 30 mM lithium because that concentration resulted in a very large stimulation of glucose transport activity. Lithium at lower concentrations also potentiated the stimulation of 2-DG uptake by 5 µU/ml of insulin (stimulatory effect of insulin was twofold higher in the presence of 10 mM lithium; data not shown).

Effect of Lithium on IP Production

To determine whether lithium inhibits IMPase in our isolated rat adipocyte preparation, changes in cellular IPs were directly measured after treatment with lithium. IP₁, IP₂, and IP₃ are all present in adipocytes (Fig. 3), with IP₁ being the predominant form (90%).
The high ratio of IP$_1$ to IP$_2$ plus IP$_3$ is similar to that reported previously in adipocytes by others (11). Treatment with 30 mM lithium for 30 min significantly increased $^3$H-labeled IP$_1$ accumulation to more than double that under basal conditions (Fig. 3), whereas IP$_2$ and IP$_3$ levels were unaffected (Fig. 3, insert).

**Effect of a Prodrug of the IMPase Inhibitor on IP Production**

The effect of L-690,488, a prodrug of the IMPase inhibitor L-690,330, on IP$_1$ accumulation was also examined. The prodrug at a concentration of 50 µM significantly increased $^3$H-labeled IP$_1$ accumulation to more than double that under basal conditions (Fig. 3), whereas IP$_2$ and IP$_3$ levels were unaffected (Fig. 3, insert).

**Effect of a Prodrug of the IMPase Inhibitor on IP Production**

The effect of L-690,488, a prodrug of the IMPase inhibitor L-690,330, on IP$_1$ accumulation was also examined. The prodrug at a concentration of 50 µM significantly increased IP$_1$ and IP$_2$ levels in a time-dependent manner (Fig. 4), demonstrating that the prodrug penetrated the adipocyte cell membrane and was cleaved to the active inhibitor. A 30-min exposure to 50 µM of the prodrug increased IP$_1$ levels to about the same extent as exposure to 30 mM lithium, i.e., about a doubling from basal levels (Fig. 4). Longer exposure to the prodrug (45 min) resulted in a further substantial accumulation of IP$_1$ to about three times that seen in the basal state.

**Effect of Prodrug on Glucose Transport**

We further examined the effects of the prodrug on basal and insulin-stimulated 2-DG transport. Incubation of adipocytes with the prodrug (5 or 50 µM) for 30 min did not enhance either basal or insulin (5 µU/ml)-stimulated 2-DG transport (Fig. 5). In the same experiments, lithium (30 mM) markedly increased both basal and insulin-stimulated 2-DG transport. In fact, the prodrug at 5 and 50 µM reduced basal glucose transport activity by 10 and 28%, respectively (significantly different from control at 50 µM). Incubation of the adipocytes with L-690,488 also produced a significant (22%) decrease in insulin-stimulated 2-DG transport activity when present at 50 µM. Exposure to the prodrug (50 µM) did not alter 2-DG transport after a 90-min incubation (data not shown).
**DISCUSSION**

The present study examined the effect of lithium on glucose transport in isolated rat adipocytes and tested the hypothesis that the effect of lithium on glucose transport is mediated by inhibition of IMPase. Our strategy was to examine the effects of a selective inhibitor of IMPase in comparison with those of lithium. The results show that lithium increases basal glucose transport activity in a concentration-dependent manner in adipocytes. In comparison with the previous study by Cheng et al. (8), our adipocytes responded to lower concentrations of lithium, and the responses were much greater than previously reported: 30 mM lithium produced a 198% increase in our study vs. a 50% increase in the previous study (8).

This study provides the first evidence that lithium potentiates the response of adipocytes to other stimuli, such as insulin, as has recently been demonstrated in skeletal muscle (25). Cheng et al. (8) reported that the effects of lithium were not additive to those of insulin, but these investigators examined only a maximally effective concentration of insulin, in which an additive effect is more difficult to distinguish. When a range of insulin concentrations was examined, it became apparent that lithium has multiple effects on glucose transport in adipocytes (Fig. 2): not only does it stimulate basal transport activity, but it also induces a leftward shift in the insulin concentration response (increased insulin sensitivity).

Our results indicate that inhibition of IMPase and subsequent accumulation of IP3 could not mimic the stimulatory effect of lithium on glucose transport activity. Treatment with the prodrug of the IMPase inhibitor, L-690,488, resulted in a significant elevation of IP3 in our adipocyte preparation. Incubation of the adipocytes with 50 µM L-690,488 for 30 min doubled IP3 levels, which is equivalent to the effect of 30 mM lithium. Lithium induced a significant increase in glucose transport activity; whereas the prodrug did not. These results suggest that accumulation of IP3 after inhibition of IMPase is not sufficient to mimic the stimulatory effect of lithium on glucose transport activity. However, we cannot rule out two possibilities. First, the IMPase inhibitor has a slightly different effect from that of lithium, because the IMPase inhibitor elevated IP2 level, but lithium did not. The increase in IP2 may somehow offset the effect of elevated IP3 to stimulate glucose transport. However, we are unaware of any precedent for IP2 to inhibit the activation of glucose transport. The second possibility is that we cannot rule out is that lithium treatment increases IP1 in a different cellular compartment from that stimulated by the IMPase inhibitor.

Recent studies by Epps-Fung et al. (10) suggested that phospholipase C (PLC) may play a role in GLUT-4-mediated glucose transport in differentiated 3T3-L1 adipocytes. This hypothesis was supported primarily through experimental interventions that reduced PLC activity. For example, the PLC inhibitor U-73122 reduced epidermal growth factor (EGF)- and insulin-stimulated glucose transport activity and GLUT-4 translocation significantly. The authors speculated that PLC or resultant products, such as IPs, play either a permissive role or mediate the stimulation of GLUT-4 translocation by insulin and EGF. Given that 1) insulin-stimulated PLC only slightly (6%), yet the inhibitor reduced insulin-stimulated transport activity extensively, and 2) the inhibitor appeared to reduce GLUT-4 levels in the basal state, PLC activity may only be permissive for GLUT-4 translocation. In that case, increases in PLC products such as IPs above basal levels may not mediate further increases in transport activity. Although Epps-Fung et al. showed that lithium increased glucose transport in the 3T3-L1 adipocytes, they did not report effects of lithium on IP levels. In our studies, we examined only conditions that increase IP levels, so we cannot conclude whether basal levels of these metabolites play a permissive role in maintaining glucose transport activity.

The effects of lithium on glucose metabolism are not limited to glucose transport. Lithium has also been shown to stimulate glycogen synthase activity in skeletal muscle (14), adipocytes (9), and hepatocytes (18). Recently, Klein and Melton (16) showed that lithium regulates cell fate determination in diverse organisms through selective and potent inhibition of glycogen synthase kinase-3 (GSK-3) (16). Therefore, lithium may activate glycogen synthase activity through inhibition of GSK-3, which inactivates glycogen synthase by phosphorylation of the enzyme.

Lithium treatment has also been shown to inhibit adenylate cyclase activation by β-adrenergic agonists and histamine in neuronal cells (12). Lithium suppresses hormone-induced formation of cAMP in a number of tissues, including the thyroid, kidney, and platelets (12). However, lithium has no effect on basal or epinephrine-induced increases in cAMP levels in muscle, a tissue in which lithium blunts the activation of glycogen phosphorylase by epinephrine and significantly stimulates glucose transport activity (25). Therefore, cAMP does not seem to be a mediator of the lithium effect on glucose transport, at least in skeletal muscle.

Lithium has been shown to modulate GTP-binding proteins. Lithium at therapeutic concentrations completely blocks both adrenergic and cholinergic agonist-induced increases in GTP binding in membranes from rat cerebral cortex (3). These results suggest that G proteins may act as the molecular site of action for both the antimanic and antidepressant effects of lithium in supporting an alternative adrenergic-cholinergic balance hypothesis of bipolar disorders (15). Recently, it has been reported that lithium stimulates rat pancreatic β-cell replication that is mediated by pertussis toxin-sensitive GTP-binding proteins (24). Interestingly, it has been demonstrated that nonhydrolyzable GTP analogs mimic effects of insulin on glucose transporter recruitment to the cell surface in permeabilized adipocytes (4). There is no direct evidence that lithium alters insulin signaling or enhances translocation of
glucose transporters in response to insulin. However, given that lithium potentiates the response to muscle contractions/hypoxia, it seems likely that lithium alters steps common to glucose transport pathways activated by these stimuli (25). A more complete understanding of the molecular basis of lithium action on glucose transport awaits further investigation.

In conclusion, our results demonstrate that lithium stimulates basal glucose transport activity and potentiates insulin-stimulated glucose transport activity in isolated rat adipocytes. However, the stimulatory effect of lithium on glucose transport in adipocytes is not due to an inhibition of IMPase and the resulting accumulation of IP₁.

Wethank Jeanne Sebaugh for the statistical analysis of the data in this study.

Received 2 December 1997; accepted in final form 28 April 1998.

REFERENCES


