Stimulatory effect of lithium on glucose transport in rat adipocytes is not mediated by elevation of IP₁

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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 IP₁. 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 (IP₁). We compared 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-[¹⁴C]glucose (2-DG) transport (10 μM 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 IP₁ accumulation. In contrast, L-690,488 increased IP₁ 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 IP₁ in rat adipocytes. The effects of lithium to stimulate glucose metabolism can be demonstrated in isolated tissues. Acute exposure to lithium has been shown to increase glucose uptake in isolated rat diaphragm muscle (14) and adipocytes (8) as well as glycogen synthesis in rat hepatocytes (18), diaphragm muscle (14), and adipocytes (8). Furthermore, the effect of lithium on glucose transport in skeletal muscle mimics the persistent effects of exercise rather than those of insulin, suggesting that a more distal step beyond the insulin receptor is involved in the actions of lithium (25).

Despite this wealth of descriptive information regarding lithium effects on glucose metabolism, the mechanisms underlying this action are poorly understood. One possibility is that these effects depend on the inhibitory action of lithium on IMPase. Recent work has identified potent and selective bisphosphonate inhibitors of IMPase (1). The further development of an ester prodrug (L-690,488) that is cell permeable and converted by the action of esterases to an active inhibitor (2) makes possible the selective inhibition of IMPase in intact cells. The prodrug L-690,488 has been shown to elevate the accumulation of IP₁ in isolated cells and brain slices (2).

The present work examined the hypothesis that lithium stimulation of glucose transport activity is mediated by inhibition of IMPase. Isolated adipocytes were used for this purpose because 1) the isolated adipocyte preparation is an excellent model system for studying glucose transport and glucose metabolism, 2) lithium has previously been shown to stimulate glucose transport in this preparation, and 3) the likelihood of inhibitor penetration into isolated cells is greater than in intact skeletal muscle preparations. Therefore, the purpose of the present study was two-fold: 1) to characterize the effects of lithium on glucose transport activity and inositol phosphate levels and 2) to determine whether the effects of lithium can be mimicked by inhibition of IMPase by the use of a commercially available prodrug of a bisphosphonate inhibitor of IMPase. Our results suggest that lithium-stimulated glucose transport is not mediated by IMPase inhibition in isolated rat adipocytes.

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, 2 H2O, and pH 7.4 MgSO4·7 H2O) containing 3% triton X-100 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.

Glucose Transport Studies

The assay for glucose transport was modified from the method described by Olefsky (19) for adipocyte incubation and uptake of 2-deoxy-ß[14C]glucose (2-DG). 2-DG is transported across the cell membrane and phosphorylated like glucose, although it cannot be further metabolized (27). The cell suspension was divided into aliquots (180 μl sample of 2.5 × 10^6 cells·1 ml⁻¹). Triplicate or quadruplicate samples were distributed to 12 × 75-mm polypropylene tubes containing 90 μl KRP buffer with or without the addition of lithium, IMPase inhibitor, or insulin. Because addition of lithium slightly alters the osmotic strength of the incubation medium, the nonpenetrating solute mannitol was added at a level sufficient to match the extra osmolarity resulting from lithium. This ensured that osmolarity was constant across all experimental groups and did not contribute to any observed effects of lithium treatment. After the treatments under the experimental groups and did not contribute to any observed effects of lithium treatment. After the treatments under the experimental conditions for the designated time periods as described in Figs. 1–5, the transport assay was initiated by adding 30 μl of 2-DG with a final specific activity of 6 μCi/μmol (at a final 2-DG concentration of 0.1 mM).

The transport assay was terminated by transferring a 200-μl aliquot of cell suspension into small polyethylene microcentrifuge tubes containing 100 μl dinonyl phthalate. The cells and the buffer were rapidly separated by centrifugation at 14,000 rpm for 8 s. Tubes were then cut through the oil layer. The cells (the upper layer) were transferred to scintillation vials, scintillation cocktail (EcoLite) was added, and radioactivity was determined by scintillation counting. The residual amount of sugar trapped in the extracellular water was determined by nonpenetrating marker [14C]mannitol, as described by Gliemann et al. (13). The extracellular space volume was ~0.07% of total space. All 2-DG uptake data were corrected for this factor. Data were normalized to the dry weight of cells, determined for each cell preparation (with weight of buffer subtracted). The dry weight of cells was routinely 20–30 mg per ml for cell suspensions used for glucose transport assay.

Uptake of 2-DG was markedly stimulated by insulin, with a maximum response of ~20-fold over basal (e.g., see Fig. 2A), and half-maximal activation (EC50) was observed at ~10 μM (data not shown). The transport assay was terminated after a 15-min incubation with 2-DG, conditions under which maximally effective insulin-stimulated 2-DG uptake was linear.

Both basal and insulin-stimulated 2-DG transport was dramatically attenuated by cytochalasin B, an inhibitor of glucose transporters (e.g., 50 μM cytochalasin B reduced maximally effective insulin-stimulated 2-DG uptake by >98%).
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. IP₃, IP₂, and IP₁ are all present in adipocytes (Fig. 3), with IP₁ being the predominant form (90%).
The high ratio of IP1 to IP2 plus IP3 is similar to that reported previously in adipocytes by others (11). Treatment with 30 mM lithium for 30 min significantly increased 3H-labeled IP1 accumulation to more than double that under basal conditions (Fig. 3), whereas IP2 and IP3 levels were unaffected (Fig. 3, inset).

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 IP1 accumulation was also examined. The prodrug at a concentration of 50 µM significantly increased 3H-labeled IP1 accumulation to more than double that under basal conditions (Fig. 4), whereas IP2 and IP3 levels were unaffected (Fig. 3, inset).

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 IP$_3$ 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 IP$_3$ in the adipocyte preparation. Incubation of the adipocytes with 50 µM L-690,488 for 30 min doubled IP$_3$ 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 IP$_3$ 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 IP$_3$ level, but lithium did not. The increase in IP$_3$ may somehow offset the effect of elevated IP$_3$ to stimulate glucose transport. However, we are unaware of any precedent for IP$_3$ to inhibit the activation of glucose transport. The second possibility that we cannot rule out is that lithium treatment increases IP$_3$ 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

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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 IP$_1$.

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REFERENCES