

# Lithium Chloride Does Not Inhibit the Proliferation of L6 Myoblasts by Decreasing Intracellular Free Inositol<sup>1</sup>

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**ABSTRACT:** We conducted a series of experiments to determine whether lithium chloride (LiCl) inhibited the proliferation of L6 myoblasts by reducing the availability of intracellular free inositol. After the myoblasts were plated in DMEM + 10% fetal bovine serum (FBS) for 24 h, medium was replaced with DMEM + 10% FBS containing 0 (control), 5, 10, or 20 mM LiCl. Cell number, protein content, and [<sup>3</sup>H]thymidine incorporation into DNA were determined at 24-h intervals. Control cells exhibited a 3.8-fold increase in cell number by 96 h in culture. Although 5 mM LiCl did not affect the rate or extent of proliferation, 10 and 20 mM LiCl caused 36 and 86% decreases, respectively ( $P < .05$ ), in cell number by 96 h in culture. The effects of LiCl could not be overcome by the addition of free inositol (up to 20 mM) to the medium. Lithium chloride caused 4.6- and 7.3-fold increases ( $P < .05$ ) in lactate dehydrogenase activity in culture media after 96 h of

exposure to 10 and 20 mM LiCl, respectively, indicating loss of viability after chronic treatment. However, the acute effects of LiCl after 24 h of treatment were reversible, as indicated by a rapid resumption of proliferation following removal of LiCl. Concentrations of 5, 10, and 20 mM LiCl caused 4.7-, 8.2-, and 9.1-fold increases ( $P < .05$ ), respectively, in the accumulation of [<sup>3</sup>H]inositol within the inositol monophosphate pool. Treatment of cells with 10 and 20 mM LiCl also increased ( $P < .05$ ) label recovered as inositol bisphosphate. Rather than depress phosphoinositide synthesis, the addition of 10 and 20 mM LiCl dose-dependently increased ( $P < .05$ ) the incorporation of [<sup>3</sup>H]inositol into phosphatidylinositol and phosphatidylinositol-4-phosphate. These results indicate that LiCl does not decrease proliferation of L6 myoblasts via a depletion in the intracellular free inositol pool. Instead, LiCl may block the hydrolysis of phosphatidyl inositides.

Key Words: Cell Cultures, Muscles, Myo-inositol, Phosphatidylinositols, Lithium Chloride

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## Introduction

Koohmaraie (1987) first reported that LiCl attenuated proliferation of L6 myoblasts, concurrent with reductions in total inositol phosphates and phosphatidyl inositols. The most widely recognized effect of LiCl is its ability to modulate the phosphatidyl inositide-mediated signal transduction system (Hallcher and Sherman, 1980; Berridge et al., 1982). Depending on cell type, Li<sup>+</sup> can enhance the agonist-induced accumulation of the inositol monophosphates, bisphosphates, and trisphosphates (Berridge et al.,

1982; Best and Malaisse, 1983; Sherman et al., 1985). This effect of Li<sup>+</sup> stems from its ability to specifically inhibit inositol monophosphate phosphatase and inositol polyphosphate 1-phosphatase (Hallcher and Sherman, 1980; Inhorn and Majerus, 1988), required for the conversion of all inositol monophosphate isomers to free inositol (Ackermann et al., 1987). Hence, the effects of Li<sup>+</sup> could be due to a decrease in the availability of free inositol for resynthesis of phosphatidyl inositol.

We have extended the results of Koohmaraie (1987) in that we have demonstrated that LiCl can inhibit the growth of L6 myoblasts within 24 h in culture. Furthermore, the effect of LiCl is dose-dependent and does not seem to result from depletion of inositol for phosphatidyl inositide synthesis. Unlike perotid gland cells (Downes and Michell, 1982) and cerebral cortical cells (Kendall and Nahorski, 1987), LiCl causes dose-dependent increases in phosphatidyl inositides in L6 myoblasts. In addition, the acute

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effects of LiCl are rapidly reversible, but chronic treatment with LiCl is associated with a decrease in cell viability.

### Materials and Methods

**Source of Materials.** Dulbecco's modified Eagle's medium (**DMEM**) and fetal bovine serum (**FBS**) were from Gibco (Gaithersburg, MD). Tissue culture flasks and dishes were from Corning (Corning, NY). Silica gel 60 HL plates were purchased from E. Merck (Darmstadt, Germany). Standards (L- $\alpha$ -phosphatidylinositol, L- $\alpha$  phosphatidylinositol 4-monophosphate, and L- $\alpha$ -phosphatidylinositol 4,5-bisphosphate) were from Sigma Chemical (St. Louis, MO). Radioisotopes [methyl- $^3\text{H}$ ]thymidine (specific radioactivity 20 Ci/mmol) and [2- $^3\text{H}$ ]myo-inositol (specific activity 14 Ci/mmol) were from Dupont-NEN (Boston, MA).

**Culture Conditions.** The L6 myoblasts were maintained in DMEM + 10% FBS (vol/vol). In experiments employing Li<sup>+</sup> or Na<sup>+</sup>, 100 mM stock solutions were prepared by addition of LiCl or NaCl to DMEM and diluted to working concentrations of 5, 10, or 20 mM. For proliferation experiments, cells were plated at  $1 \times 10^5$  cells/well in 12-well culture dishes in DMEM + 10% FBS. Following attachment (24 h), the medium was aspirated from wells and replaced with DMEM + 10% FBS containing 0, 5, 10, or 20 mM LiCl. Experiments investigating the specificity of LiCl action were performed with 20 mM NaCl. Unless specified otherwise, cultures were refed with identical medium daily. At 24-h intervals, triplicate monolayers/treatment were dispersed with EDTA/trypsin (.05%), and cell number was determined with a Coulter counter (Coulter Electronics, Hialeah, FL). In replicate plates, the medium was aspirated from triplicate monolayers per treatment. The cells were washed twice with PBS and solubilized in .1 M NaOH/.1% Triton X-100, and protein content of individual samples was determined (Lowry et al., 1951). To assess the effects of LiCl on DNA synthesis, cells were plated as in the proliferation experiments and allowed to attach for 24 h. The medium was aspirated, and cells were pretreated for 24 h in DMEM + 10% FBS containing 0, 5, 10, or 20 mM LiCl. The medium was removed, and similar medium containing .25  $\mu\text{Ci/mL}$  [ $^3\text{H}$ ]thymidine was added. After an 18-h pulse, the monolayer was rinsed twice with cold PBS, twice with cold 5% trichloroacetic acid (**TCA**), once with cold H<sub>2</sub>O, and dissolved in .5 mL of .1 M NaOH/.1% Triton X-100. Duplicate aliquots were evaluated in a liquid scintillation counter.

In other experiments, cells were plated at  $1 \times 10^4$  cells/cm<sup>2</sup> in 25-cm<sup>2</sup> culture flasks in DMEM + 10% FBS. Following attachment at 24 h, medium was aspirated from flasks and replaced with DMEM + 10% FBS containing 0, 10, or 20 mM LiCl, and cells were

incubated for 24 h (pretreatment). The medium was aspirated and cells were washed with DMEM and incubated for 1 h in DMEM + .1% FBS (vol/vol) to remove residual LiCl. Cells were then refed with DMEM + 10% FBS containing 0, 10, or 20 mM LiCl. Cell numbers were determined at 24 and 48 h following LiCl removal, and the percentage change in cell number for each 24-h period was calculated. The effect of LiCl on cell viability was determined by measuring lactate dehydrogenase activity in culture media of treated cells with a commercially available kit (Sigma Chemical) per manufacturer's directions.

**Inositol Phosphate Metabolism.** Cells were plated at  $1 \times 10^4$  cells/cm<sup>2</sup> in 60- $\times$  15-mm culture dishes in DMEM + 10% FBS. Following attachment at 24 h, the medium was aspirated and replaced with DMEM + 10% FBS containing 5  $\mu\text{Ci/mL}$  [ $^3\text{H}$ ]inositol. Cultures were incubated for 48 h to allow labeling of the inositol phosphate pools. The medium was aspirated, and cells were washed twice with PBS and refed with DMEM + 10% FBS containing 0, 5, 10, or 20 mM LiCl. At 15, 30, 60, or 120 min following the addition of LiCl, the medium was aspirated and cultures were terminated by the addition of 10% TCA. Dishes were placed on ice for 30 min to allow complete extraction of the water-soluble inositol phosphates. The TCA extract was removed, the cellular precipitate was washed twice with H<sub>2</sub>O, and the washings were combined with the initial TCA extract. The combined TCA extract was neutralized with 2 N NaOH and washed three times with diethyl ether. Inositol phosphate pools were separated essentially as described previously (Berridge et al., 1982). Briefly, samples were loaded onto pre-equilibrated columns of AG1-X8 anion exchange resin (BioRad Lab., Richmond, Ca.) and washed four times with 3 mL of H<sub>2</sub>O to elute free inositol. Inositol mono-, bis-, and triphosphate pools were eluted sequentially by the stepwise addition of six 3-mL aliquots of .2, .4, and 1.0 M ammonium formate in .1 M formic acid. Individual fractions were eluted directly into scintillation vials, and radioactivity in individual fractions was determined by liquid scintillation counting. Cell number was determined in parallel plates.

**[ $^3\text{H}$ ]Inositol Incorporation into Phosphatidyl Inositols.** Cells were plated at  $1 \times 10^4$  cells/cm<sup>2</sup> in 25-cm<sup>2</sup> flasks in DMEM + 10% FBS and cultures were incubated for 48 h. The medium was aspirated and replaced with DMEM + 10% FBS containing 5  $\mu\text{Ci/mL}$  [ $^3\text{H}$ ]inositol and 0, 5, 10, or 20 mM LiCl. At 6, 12, 24, or 48 h following the addition of radioisotope, cellular lipids were extracted with the method of Folch et al. (1957). Lipids were redissolved in 100  $\mu\text{L}$  chloroform/methanol (3:1; vol/vol) and stored at -80°C. Phosphatidyl inositols were separated by one-dimensional thin layer chromatography (**TLC**) on preactivated Silica gel HL TLC plates. Samples and standards (phosphatidyl inositol [**PI**], phosphatidyl inositol 4-phosphate [**PI(4)P**], and phosphatidyl inositol

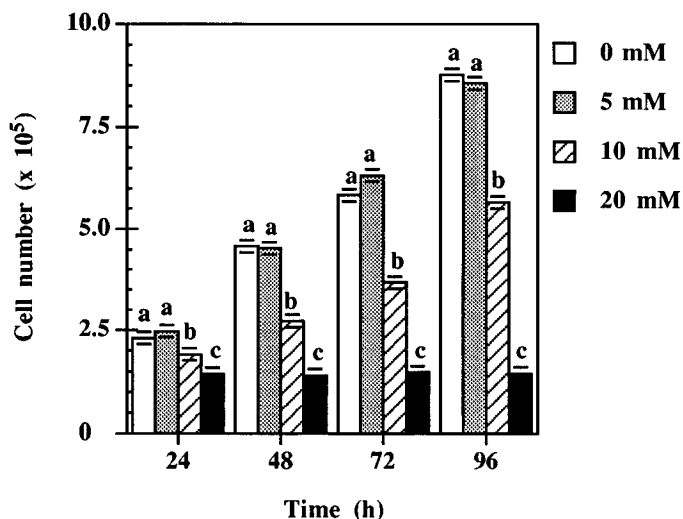


Figure 1. The effect of LiCl on cell number in myoblast cultures over time. Cells were plated in 12-well plates ( $1 \times 10^5$  cells/well) and allowed to attach. The medium was aspirated and replaced with DMEM + 10% FBS containing 0, 5, 10, or 20 mM LiCl. Cell number was determined at 24-h intervals (up to 96 h) following the addition of treatments. Columns represent the least squares means  $\pm$  SEM from three replicate experiments. <sup>a,b,c</sup>Within a time point, columns with different superscripts differ ( $P < .05$ ).

4,5-bisphosphate [PI(4,5)P<sub>2</sub>]) were spotted onto individual lanes. Separation of phosphatidyl inositol isomers was achieved using a solvent system composed of chloroform-methanol-concentrated NH<sub>4</sub>OH-H<sub>2</sub>O (45:45:4:10; vol/vol). Lipid bands were visualized by exposure to I<sub>2</sub>, and those corresponding to PI, PI(4)P, and PI(4,5)P<sub>2</sub> were scraped into separate scintillation vials. Radioactivity in individual bands was determined by liquid scintillation counting. Cell number for individual treatments and times was determined in parallel flasks.

**Statistical Analysis.** Individual experiments were replicated at least three times. The data were analyzed using the GLM procedures of SAS (1989). Sources of variation included experiment, treatment, time, and their interaction. Data are presented as the least squares means  $\pm$  SEM. Differences between means, resulting from time in culture or treatment, were determined by Fisher's Least Significant Difference test with comparison of  $P < .05$  considered significant.

## Results

**L<sub>6</sub> Myoblast Proliferation.** The addition of LiCl to the culture medium resulted in a dose-dependent inhibition of myoblast proliferation (Figure 1). Control myoblasts proliferated rapidly, as indicated by a

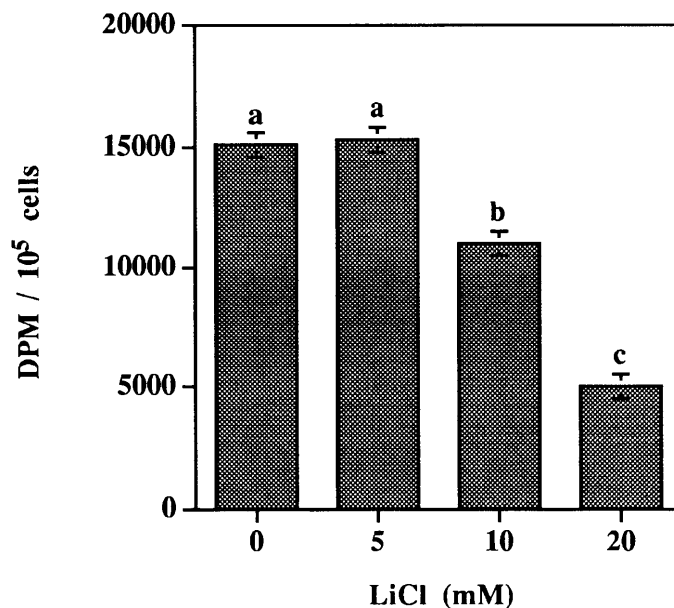


Figure 2. The effect of LiCl on the incorporation of [<sup>3</sup>H]thymidine into the DNA of myoblast cultures. Cells were plated in 12-well plates ( $1 \times 10^5$  cells/well) and allowed to attach. The medium was aspirated and cultures pretreated for 24 h with DMEM + 10% FBS containing 0, 5, 10, or 20 mM LiCl. The medium was subsequently removed and replaced with similar medium containing .25  $\mu$ Ci/mL [<sup>3</sup>H]thymidine. Cultures were incubated an additional 18 h and incorporation of radioisotope determined. Columns represent the least squares means  $\pm$  SEM from three replicate experiments. <sup>a,b,c</sup>Columns with different superscripts differ ( $P < .05$ ).

3.8-fold increase ( $P < .05$ ) in cell number from 24 to 96 h in culture. Myoblast proliferation in the presence of 5 mM LiCl was not different ( $P > .05$ ) from control cultures over time. In contrast, 10 mM LiCl caused 41, 37, and 36% ( $P < .05$ ) reductions in cell number by 48, 72, and 96 h, respectively. Treatment of cells with 20 mM LiCl caused complete cessation ( $P < .05$ ) of cell proliferation within 24 h after addition.

The effects of LiCl on proliferation were supported by the measurement of DNA synthesis (Figure 2). A 24-h pretreatment of L6 myoblasts with 10 or 20 mM LiCl caused 17 and 65% decreases ( $P < .05$ ), respectively, in radioisotope recovered in the acid-precipitable (DNA) fraction of myoblasts. As observed for cell number, 5 mM LiCl did not affect ( $P > .05$ ) the incorporation of [<sup>3</sup>H]thymidine into DNA. The inhibition of myoblast proliferation seemed to be specific for Li<sup>+</sup>, because in cultures treated with 20 mM NaCl, differences were detected in neither the rate nor extent of proliferation ( $9.74 \pm .31$  vs  $9.64 \pm .03 \times 10^5$  cells by 96 h in culture for control and 20 mM NaCl treatments, respectively;  $P > .10$ ). In addition, the LiCl-induced inhibition of myoblast proliferation could not be overcome by the addition of 10 mM inositol to the culture medium (data not shown).

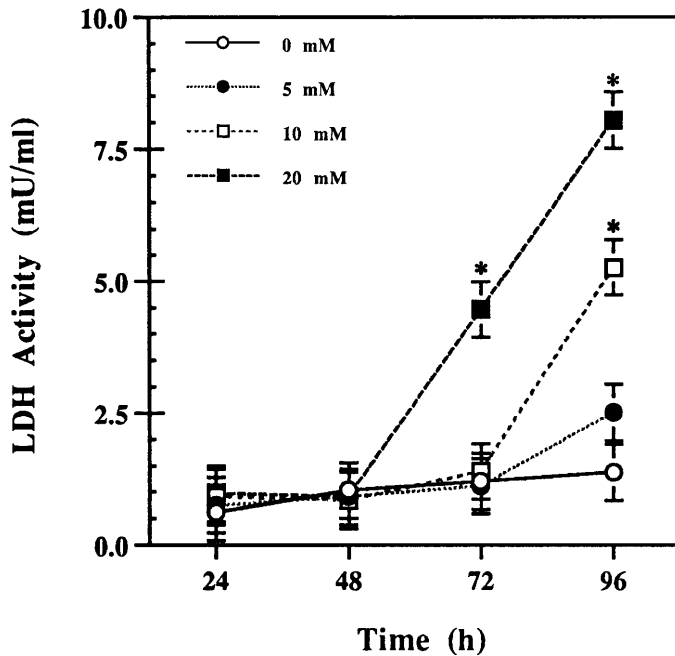


Figure 3. The effect of LiCl on cell viability as determined by measurements of lactate dehydrogenase (LDH) activity in culture media. Cells were plated in 12-well plates ( $1 \times 10^5$  cells/well) and allowed to attach. The medium was aspirated and replaced with DMEM + 10% FBS containing 0, 5, 10, or 20 mM LiCl. The activity of LDH in culture medium was determined at 24-h intervals (up to 96 h) following the addition of treatments. Data points represent the least squares means  $\pm$  SEM from three replicate experiments. \*Treatments differ from 0 mM LiCl ( $P < .05$ ).

Lithium chloride caused a dose- and time-dependent increase ( $P < .05$ ) in lactate dehydrogenase activity in the culture media (Figure 3). For 20 mM LiCl, this increase was apparent by 72 h in culture. Maximal increases of 4.6- and 7.3-fold occurred at 96 h in culture for the 10 and 20 mM LiCl treatments, respectively. Removal of LiCl from the medium after 24 h of treatment allowed cells to resume proliferation completely (Figure 4). Cells pretreated with 10 mM LiCl and allowed to recover (10 mM [R]) displayed the same ( $P > .05$ ) relative change in cell number as control cells within 24 h of LiCl removal. Cell proliferation also resumed in cultures pretreated with 20 mM LiCl (20 mM [R]), but the time course for recovery was delayed.

**L6 Myoblast Protein Content.** By 48 h after treatment, cells cultured with 5 mM LiCl contained a 14% greater ( $P < .05$ ) concentration of protein than control cultures (Figure 5). Treatment with 20 mM LiCl elicited a 29% increase ( $P < .05$ ) in protein content by 24 h and a maximal increase of 117% by 48 h. Similarly, 10 mM LiCl induced a maximal increase ( $P < .05$ ) in protein concentration of 41% by 72 h. The increase in protein concentration coincided with alter-

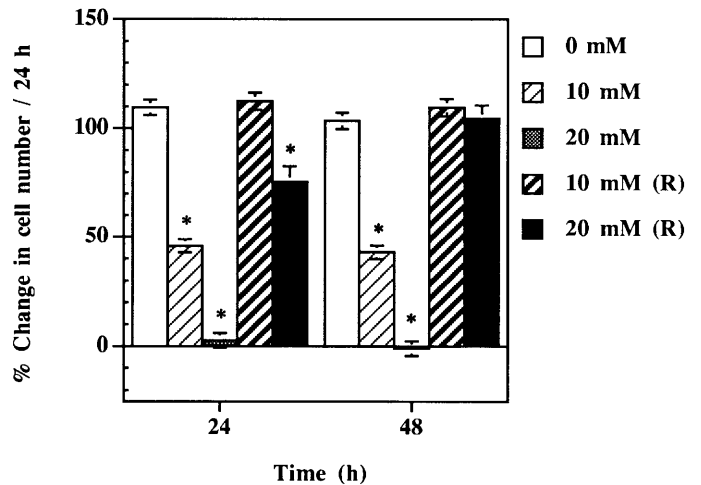


Figure 4. The recovery of myoblast cultures from LiCl treatment. Cells were plated in T-25 flasks (10,000 cells/cm<sup>2</sup>) and allowed to attach. The medium was aspirated, and cells were pretreated for 24 h with DMEM + 10% FBS containing 0, 10, or 20 mM LiCl. Cultures were subsequently refed with identical medium (0, 10, or 20 mM LiCl treatments) or with DMEM + 10% FBS [10 mM (R) and 20 mM (R)]. Cell number was determined at 24-h intervals (24 and 48 h) following the removal of LiCl. Columns represent the least squares means  $\pm$  SEM from three replicate experiments. \*Within a time point, treatments differ from 0 mM LiCl ( $P < .05$ ).

ations in cell morphology; cells treated with 10 and 20 mM LiCl were much larger than control cells, as judged by phase contrast microscopy.

**Accumulation of Inositol Phosphates.** Time in culture did not substantially alter the recovery of label in inositol phosphates control cultures (Figure 6). For control cultures,  $54 \pm 1\%$  of the [<sup>3</sup>H]inositol was recovered as inositol monophosphate (IP), and the inositol bisphosphate (IP<sub>2</sub>) and inositol trisphosphate (IP<sub>3</sub>) pools accounted for  $9 \pm 1$  and  $37 \pm 1\%$ , respectively, of the recovered label. Consistent with its inhibition of inositol monophosphate phosphatase (Hallcher and Sherman, 1980), LiCl caused a dose-dependent increase ( $P < .05$ ) in label recovered as IP in L6 myoblasts within 15 min after LiCl addition (32, 73, and 94% increases vs controls for the 5, 10, and 20 mM LiCl treatments, respectively). The LiCl-induced accumulation of IP increased over time, as indicated by the 4.7-, 8.2-, and 9.1-fold increase by 120 min ( $P < .05$ ) relative to control for the 5, 10 and 20 mM LiCl treatments, respectively.

Treatment of myoblasts with LiCl also increased ( $P < .05$ ) the recovery of label in the IP<sub>2</sub> pool (Figure 6). At 10 and 20 mM LiCl, the increases in labeled IP<sub>2</sub> were apparent at 30 min (26 and 90% increases relative to control, respectively) and were sustained to 120 min. The addition of 5 mM LiCl resulted in only a small (18%), transient increase ( $P < .05$ ) in the IP<sub>2</sub>

## Discussion

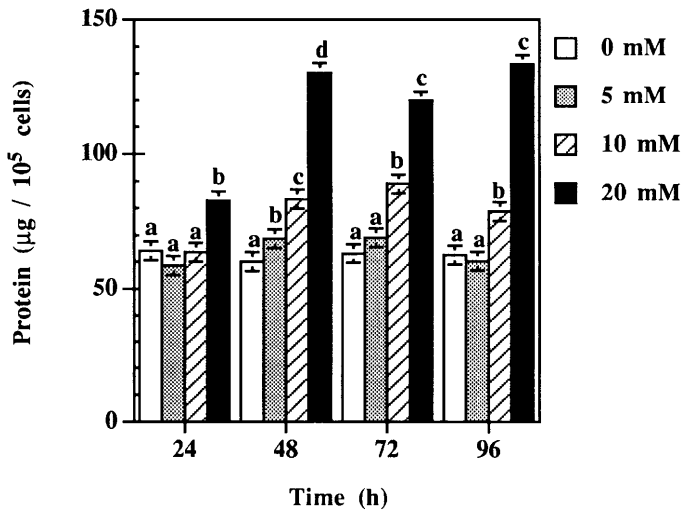


Figure 5. The effect of LiCl on the protein content ( $\mu\text{g}/10^5$  cells) of myoblast cultures over time. Cells were plated in 12-well plates ( $1 \times 10^5$  cells/well) and allowed to attach. The medium was aspirated and replaced with DMEM + 10% FBS containing 0, 5, 10, or 20 mM LiCl. Protein content of cultures was determined at 24-h intervals (up to 96 h) following the addition of treatments. Columns represent the least squares means  $\pm$  SEM from three replicate experiments. <sup>a,b,c,d</sup>Within a time point, columns with different superscripts differ ( $P < .05$ ).

pool. Except for small, transient increases ( $P < .05$ ) of 18 and 31% for 10 and 20 mM LiCl at 30 min, LiCl had negligible effects on the recovery of label as IP3.

**Incorporation of [<sup>3</sup>H]Inositol into Phosphatidyl Inositides.** Lithium chloride caused a dose-dependent increase in the incorporation of [<sup>3</sup>H]inositol into the phosphatidyl inositides (Figure 7). In control cultures, the incorporation of [<sup>3</sup>H]inositol into PI, PI(4)P, and PI(4,5)P<sub>2</sub> increased ( $P < .05$ ) 3.0-, 2.8-, and 3.9-fold, respectively, between 6 and 24 h. Addition of 5 mM LiCl to cultures had no effect on [<sup>3</sup>H]inositol incorporation into phosphatidyl inositides. Apparent isotopic equilibrium was achieved within 24 h, because similar ( $P > .05$ ) amounts of label were recovered within each of the phosphatidyl inositide pools at 24 and 48 h for the 0 and 5 mM LiCl treatments. The 10 and 20 mM LiCl treatments increased ( $P < .05$ ) incorporation of [<sup>3</sup>H]inositol into PI, PI(4)P, and PI(4,5)P<sub>2</sub>. At 10 mM LiCl, 30 and 65% increases in label in PI were observed by 24 and 48 h, respectively. Addition of 20 mM LiCl caused a 64% increase in labeled PI within 12 h, and a 152% increase by 48 h. In myoblasts treated with 10 mM LiCl, increases ( $P < .05$ ) in PI(4)P (77%) and PI(4,5)P<sub>2</sub> (62%) were apparent within 48 h. Similarly, 20 mM LiCl resulted in 108% and 78% increases in labeled PI(4)P and PI(4,5)P<sub>2</sub>, respectively, by 48 h.

The monovalent cation, Li<sup>+</sup>, has been reported to modulate the proliferation and/or growth of a number of cell types in vitro (e.g., Gallicchio and Chen, 1981; Rybak and Stockdale, 1981). Koohmaraie (1987) reported that the addition of 10 mM LiCl to L6 myoblasts caused an approximate 50% reduction in cell number 48 h after treatment. The present study indicates that the inhibition of myoblast proliferation is specific to the Li<sup>+</sup> ion and is concentration-dependent. Consistent with Koohmaraie (1987), the inhibition of myoblast proliferation by LiCl was supported by a measurement of DNA synthesis, with only those concentrations (10 and 20 mM) that inhibited myoblast growth depressing [<sup>3</sup>H]thymidine incorporation into DNA.

The increase in protein concentration and cell size induced by LiCl were similar to results reported previously (Koohmaraie, 1987). Changes in cell morphology caused by LiCl treatment also have been reported for melanoma cells (Nordenberg et al., 1987) and HL60 cells (Tyobeka and Becker, 1990). Madiehe et al. (1995) reported that treatment of HL60 cells with greater than 10 mM LiCl decreased cell proliferation, but, in contrast to results from this study, it was accompanied by cytoplasmic and nuclear condensation.

Lithium has been reported to modulate a number of intracellular processes in vitro, such as ion transport, cyclic nucleotide metabolism, G protein function, and the activity of DNA polymerase (Gelfand et al., 1979; Hart, 1979; Avissar et al., 1988; Drummond, 1988; Ziaie and Kefalides, 1989). Perhaps most widely recognized is the ability of Li<sup>+</sup> to modulate the phosphatidyl inositide-mediated signal transduction system (Hallcher and Sherman, 1980). Koohmaraie (1987) previously reported that LiCl decreased total inositol phosphates in myoblasts. However, on a per cell basis, recovery of labeled inositol in inositol phosphates was not affected (Koohmaraie, 1987). In contrast, we demonstrated increased IP and IP<sub>2</sub> during acute (15- to 120-min) exposure to 10 and 20 mM LiCl. The results of Koohmaraie (1987) suggest that intracellular inositol phosphate pools may return to normal after 48 h of exposure to LiCl.

The pronounced increase in IP is consistent with work with other cell or tissue types (Berridge et al., 1982; Best and Malaisse, 1983; Sherman et al., 1985; Ackermann et al., 1987), and it indicates the ability of LiCl to inhibit the activity of inositol monophosphate phosphatase in myoblasts. We also observed increased label recovered in the IP<sub>2</sub> pool in LiCl-treated myoblasts, putatively due to inhibition of inositol polyphosphate 1-phosphatase by LiCl (Berridge et al., 1982; Downes and Michell, 1982; Drummond et al., 1984; Thomas et al., 1984). In contrast to the inhibitory effects of 10 and 20 mM LiCl, 5 mM LiCl had only transient effects on label recovered as IP<sub>2</sub>.

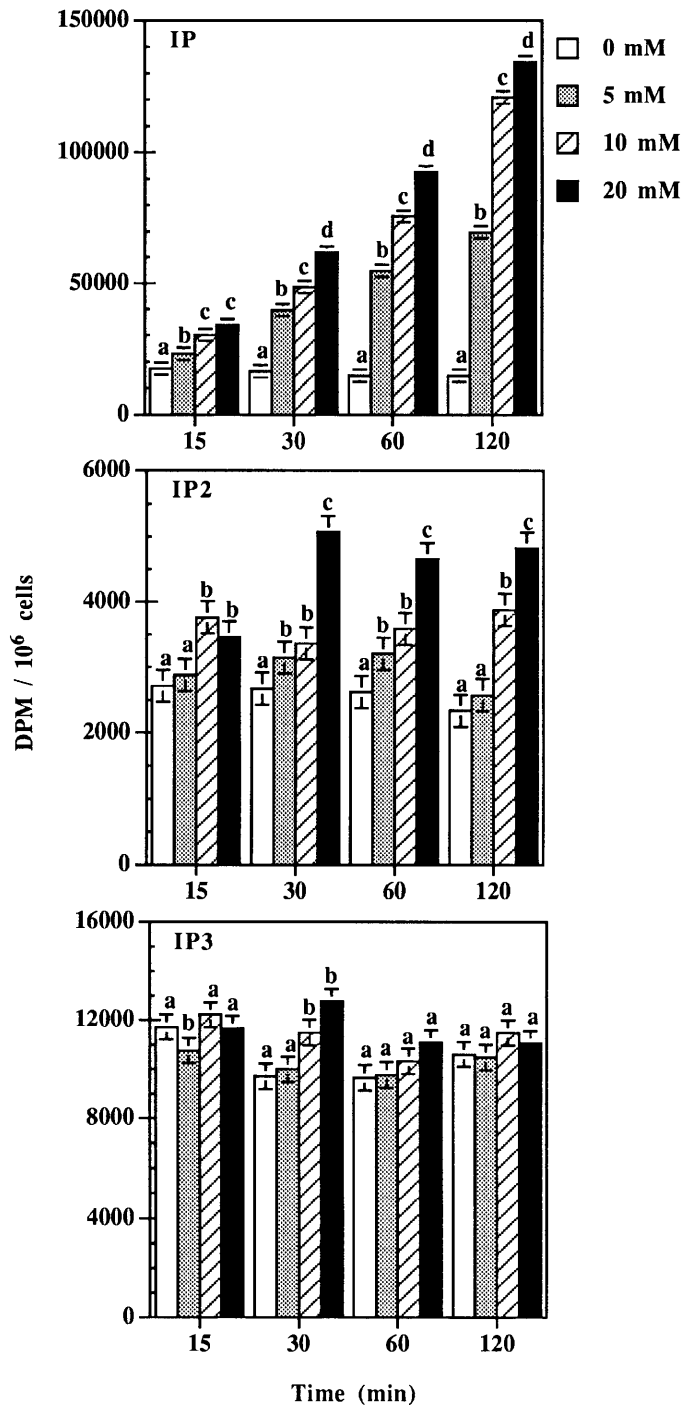


Figure 6. The effect of LiCl on  $[^3\text{H}]$ inositol phosphate accumulation in  $L_6$  myoblasts. At the indicated times inositol phosphates were extracted and separated into inositol monophosphates (IP), inositol bisphosphates (IP2), and inositol trisphosphates (IP3) as described in Materials and Methods. Columns represent the least squares means  $\pm$  SEM from three replicate experiments. <sup>a,b,c,d</sup>Within a time point, columns with different superscripts differ ( $P < .05$ ).

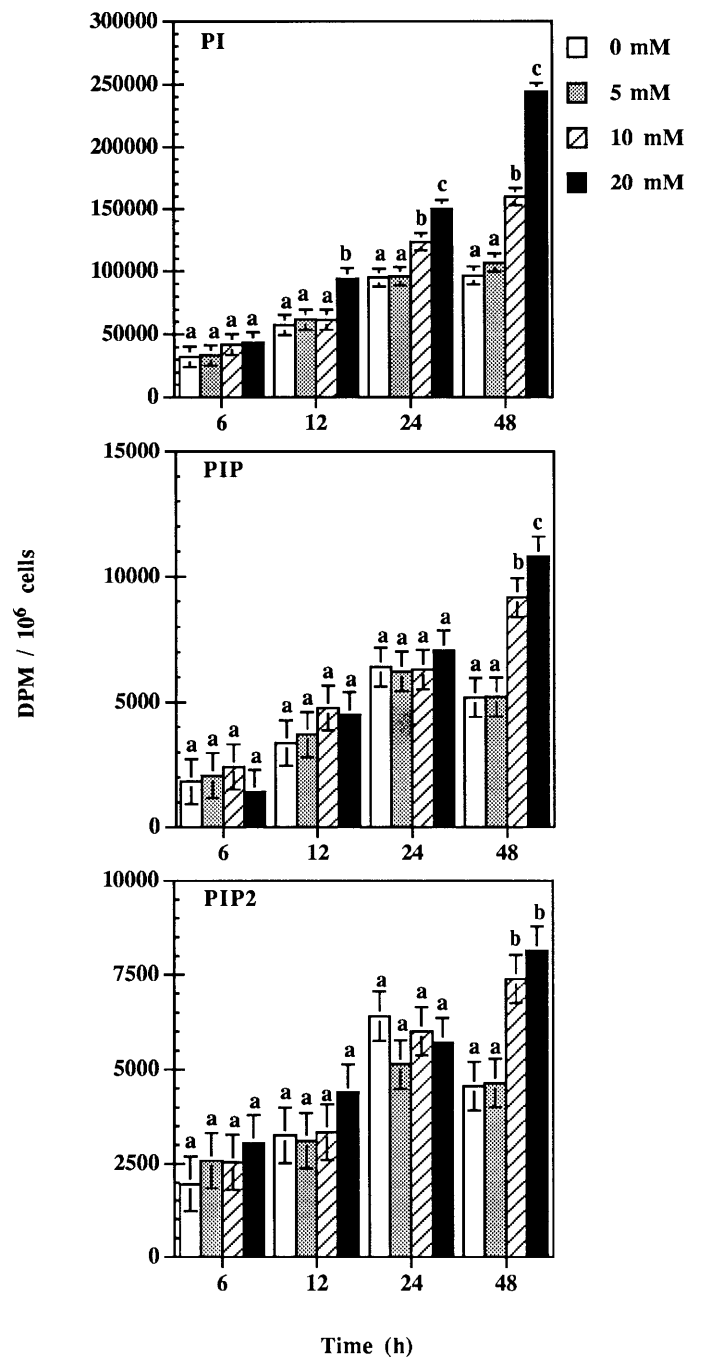


Figure 7. The effect of LiCl on the incorporation of  $[^3\text{H}]$ inositol into phosphatidyl inositides. At the indicated times, phosphatidyl inositides were extracted and separated into phosphatidyl inositol (PI), phosphatidyl inositol 4-phosphate (PIP), and phosphatidyl inositol 4,5-bisphosphate (PIP2) as described in Materials and Methods. Columns represent the least squares means  $\pm$  SEM from three replicate experiments. <sup>a,b,c</sup>Within a time point, columns with different superscripts differ ( $P < .05$ ).

Hence, inositol polyphosphate 1-phosphatase in L6 myoblasts is less sensitive to LiCl than the inositol monophosphate phosphatase, as was reported previously for hepatocytes (Thomas et al., 1984).

Unlike the time-dependent effect of LiCl on the recovery of label within the IP pool, the accumulation of label within the IP2 pool did increase with time in myoblasts treated with 5 or 10 mM LiCl. Although the basis for the differential effects on the IP and IP2 pools are not known, it suggests a shift in the metabolic pathway used for the conversion of IP3 to IP (Majerus et al., 1988). Consistent with the inability of Li<sup>+</sup> to inhibit inositol polyphosphate 5-phosphatase (Berridge et al., 1982), LiCl had negligible effects on the recovery of label as IP3 in myoblasts.

Because Li<sup>+</sup> inhibits inositol monophosphate phosphatase, decreased myoblast proliferation caused by Li<sup>+</sup> may result indirectly from a decrease in free inositol available for resynthesis of phosphatidyl inositides. Support for this hypothesis primarily has been derived from reports of elevated I(1)P and decreased free inositol in the neural tissue of rats treated with Li<sup>+</sup> (Allison and Stewart, 1971; Sherman et al., 1981, 1986). In addition, several studies have documented a reduction in the levels of the phosphatidyl inositides in cells chronically stimulated with LiCl (Downes and Michell, 1982; Drummond, 1987; Kendall and Nahorski, 1987). Myoblasts seem to respond differently to treatment with LiCl. Koohmaraie (1987) demonstrated no effect (on a per-cell basis) of LiCl on the accumulation of labeled inositol in phosphatidyl inositols, and, in the present investigation, Li<sup>+</sup> enhanced, in a time- and dose-dependent manner, the incorporation of [<sup>3</sup>H]inositol into phosphatidyl inositides in myoblasts. Although the basis for the differences reported here and previously (Koohmaraie, 1987) is unknown, these results suggest that myoblasts remaining after LiCl treatment can effectively take up and utilize inositol from the culture media. In conjunction with the inability of supplemental inositol to overcome the inhibition of myoblast proliferation by LiCl, these data indicate that the effects of LiCl on myoblast proliferation were not caused by a depletion of free inositol.

It has been demonstrated that LiCl can induce cell cycle arrest (at the G2/M interface) and apoptosis in HL60 cells (Madiehe et al., 1995); consistent with results from this study, the effects on measurements of cell viability were apparent only after chronic (i.e., 48-h) treatment. However, the morphological changes in HL60 cells (cytoplasmic and chromatin condensation) were different from the effects we observed (i.e., increased protein content and cell size). The considerable delay between inhibition of proliferation (24 h) and increase in media lactate dehydrogenase activity (72 h) indicates that the reduction in proliferation was not the result of decreased cell viability during the first 48 h. Future studies will be necessary to

address whether LiCl inhibits myoblast proliferation via cell cycle arrest or apoptosis.

## Implications

The results of this investigation provide two independent results that indicate that lithium chloride (LiCl) does not decrease myoblast proliferation by decreasing intracellular free inositol: the addition of inositol to the media did not overcome the reduction in proliferation, and phosphatidyl inositides accumulated instead of being depleted in the presence of LiCl. Thus, LiCl may affect myoblast proliferation by depressing the hydrolysis of phosphatidyl inositides. This implies that mitogens in the culture media stimulate myoblast proliferation via the phosphatidyl inositide signal transduction pathway.

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