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# Lithium chloride at a therapeutic concentration reduces $Ca^{2+}$ response in protein kinase C down-regulated human astrocytoma cells

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**Abstract**

Since the therapeutic efficacy of $Li^+$ in the treatment of mood disorder is observed only after chronic administration, we examined whether long-term $Li^+$ treatment with a therapeutic concentration affected the elevation of intracellular-free $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) induced by carbachol, a muscarinic receptor agonist, in 1321N1 human astrocytoma cells. Carbachol caused $[Ca^{2+}]_i$ elevation through phosphoinositide hydrolysis in a concentration-dependent manner. Treatment of the cells with phorbol 12-myristate 13-acetate (PMA), a protein kinase C activator, for 2 min resulted in a reduction of the carbachol-induced $[Ca^{2+}]_i$ elevation. However, PMA did not reduce the carbachol-induced $[Ca^{2+}]_i$ elevation in cells treated with PMA for 48 h, reflecting the down-regulation of protein kinase C. Although $Li^+$ at a therapeutic concentration (1 mM) did not affect the carbachol-induced $[Ca^{2+}]_i$ elevation in normal cells, it potently inhibited the $[Ca^{2+}]_i$ elevation in protein kinase C down-regulated cells. This inhibitory action of $Li^+$ was observed in a concentration- and time-dependent manner. When protein kinase C activity was directly determined, $Li^+$ treatment did not restore protein kinase C activity in protein kinase C down-regulated cells. [$^3$H]Quinuclidinyl benzylate, a muscarinic receptor ligand, bound to membranes derived from normal and protein kinase C down-regulated cells with a similar $K_d$ and $B_{max}$, and $Li^+$ did not affect these parameters of [$^3$H]quinuclidinyl benzylate binding. These results indicated that $Li^+$ at a therapeutic concentration reduced the muscarinic receptor-mediated increased in $[Ca^{2+}]_i$ under the protein kinase C-deficient condition without affecting muscarinic receptor or protein kinase C activity. 

*Keywords:* Lithium chloride; Bipolar mood disorder; Protein kinase C; Down regulation; 1321N1 human astrocytoma cell; $Ca^{2+}$ mobilization, intracellular

## 1. Introduction

$Li^+$ has been used for the treatment of bipolar mood disorder for more than 40 years (Schou, 1997). Many investigations have been performed to clarify the mechanism of action of $Li^+$. Since $Li^+$ inhibits inositol 1-monophosphatase and attenuates phosphatidylinositol turnover, it is assumed that the phosphatidylinositol-mediated signaling pathway is changed after $Li^+$ treatment (Moscovich et al., 1990). It is known that concentrations of $Li^+$ over 4–5 mM are necessary to inhibit phosphatidylinositol turnover (Allison and Blister, 1976). However, $Li^+$ at these concentrations causes toxic side effects in patients (Schou, 1997), and $Li^+$ at therapeutic concentrations (below 1 mM) is reported to have no effect on inositol monophosphatase activity in human cerebrospinal fluid (Atack et al., 1993).

As described above, $Li^+$ is thought to interfere with the phosphatidylinositol-mediated signaling pathway at high concentrations. In the phosphatidylinositol-mediated signaling pathway, phosphatidylinositol 4,5-bisphosphate ($PIP_2$) is cleaved by phospholipase C to yield two second messengers, inositol 1,4,5-trisphosphate ($IP_3$) and diacylglycerol (Berridge and Irvine, 1984). $IP_3$ mobilizes $Ca^{2+}$ from intracellular storage sites (Streb et al., 1983), while diacylglycerol activates protein kinase C, resulting in phosphorylation of intracellular multiple proteins (Nishizuka, 1986). Membrane-associated protein kinase C activity was found to be elevated in the platelets of subjects with mania (Friedman et al., 1993). Although this finding has not been supported

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by other investigators yet, it seems likely that protein kinase C may play a critical role in the pathogenic mechanism of mania or depression of bipolar mood disorder (Bebchuk et al., 2000). We previously have shown that protein kinase C is down-regulated by treatment with a phorbol ester for 48 h or more in human astrocytoma cells (Kurita et al., 2000). To understand the mechanism of $Li^+$ action under conditions of an altered protein kinase C activity, which is possibly implicated in the pathogenesis of bipolar mood disorder, we examined the effect of long-term $Li^+$ treatment at a therapeutic concentration on agonist-induced elevation of intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) in protein kinase C down-regulated human astrocytoma cells.

## 2. Methods

### 2.1. Cell culture

Human astrocytoma cells (1321N1) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum, 50 U/ml of penicillin and 5 μg/ml of streptomycin in a 37 °C humidified incubator in an atmosphere of 95% $O_2$ and 5% $CO_2$.

### 2.2. Measurement of intracellular $Ca^{2+}$ concentration

Human astrocytoma cells cultured on a 150-mm dish were washed three times with a modified Tyrode solution (composition, mM: NaCl 137, KCl 2.7, $MgCl_2$ 1.0, $CaCl_2$ 0.18, glucose 5.6, HEPES 10, pH 7.4). Cells were freed from the dish by treatment with 0.1% collagenase and 1.0% bovine serum albumin in 10 ml of the modified Tyrode solution for 15 min at 37 °C. Cells liberated from the dish were collected into a 50-ml tube and centrifuged at 250 × *g* for 1 min, and then they were washed once with 10 ml of the modified Tyrode solution. Cells ($1-5 \times 10^6$/ml) were treated with 1 μM fura 2-acetoxymethyl ester (AM) at 37 °C for 15 min, centrifuged at 250 × *g* for 1 min, in order to remove the remaining fura 2-AM, and then washed twice with the modified Tyrode solution. Cells were suspended in the modified Tyrode solution in concentrations of $1-5 \times 10^6$/ml, and 1–2 ml of the cell suspension was used for the fura 2 assay. Fluorescence of fura 2 at 510 nm was measured alternately at excitation waves of 340 and 380 nm, using a spectrofluorometer (Hitachi, F-2000). The maximum ratio of the fluorescence at 510 nm was obtained in the presence of 0.1% Triton X-100, and the minimum fluorescence ratio was obtained in the presence of 2 mM EGTA. $[Ca^{2+}]_i$ was calculated by using the $K_d$ of fura 2 to $Ca^{2+}$ as 224 nM.

### 2.3. Measurement of protein kinase C activity

Protein kinase C activity was determined by using a protein kinase C enzyme assay system (Amersham) according to the manufacturer's instructions with a slight modification. 1321N1 cells were cultivated on a 35-mm dish for 7 days at a density of $2 \times 10^5$ cells/ml. The cells were incubated with or without 1 μM PMA at 37 °C for 30 min in Eagle's minimum essential medium buffered with 20 mM HEPES, pH 7.4 (EMEM-HEPES). Then the cells were scraped off and homogenized by sonication in homogenization buffer (50 mM Tris/HCl, 0.3% (w/v) β-mercaptoetanol, 5 mM EDTA, 10 mM EGTA, *p*-amidinophenyl methansulfonyl fluoride 5 mg/100 ml, 10 mM benzamide, pH 7.5 measured at 4 °C). Then the homogenized cells were incubated with a substrate peptide and [γ-$^{32}$P]ATP (40 μCi/ml) for 15 min at 37 °C in a final volume of 5 μl, according to the instructions of the protein kinase C enzyme assay system, except that PMA was removed from the buffer. The substrate peptide (Arg-Lys-Thr-Leu-Arg-Arg-Leu-OH) has a phosphorylation site specific for protein kinase C. After termination of the reaction by addition of the stop regent (5 μl), 35 μl of each sample was spotted onto filter paper. The paper was washed twice for 5 min in 5% acetic acid. The radioactivity on each filter paper was counted with a liquid scintillation counter.

### 2.4. Binding studies with [$^3$H]quinuclidinyl benzylate

Following lysis of cells in 10 mM HEPES–10 mM EDTA (pH 7.4), the membranes were prepared by means of three centrifugations at 45,000 × *g* for 10 min and resuspension in 10 mM HEPES–10 mM EDTA buffer (pH 7.4). The final pellet was resuspended in 10 mM HEPES containing 5 mM $MgCl_2$ (pH 7.4). Membranes not used on the day of preparation were frozen at −80 °C in 0.32-M sucrose–5 mM $MgCl_2$–10 mM HEPES (pH 7.4). Membranes (250 μg) were incubated at 37 °C for 30 min with [$^3$H]quinuclidinyl benzylate in 5 mM $MgCl_2$–10 mM HEPES (pH 7.4) in a final volume of 200 μl. The reaction was started by addition of membranes and terminated by the addition of 4 ml of ice-cold buffer (140 mM NaCl and 10 mM Tris, pH 7.4). The membranes were trapped on a glass-fiber filter (Advantec GC-50) under reduced pressure, and the filter was washed with an additional 8 ml of the buffer. Non-specific binding of [$^3$H]quinuclidinyl benzylate was defined as the amount of binding in the presence of 1 μM atropine. All assays were carried out in triplicate.

### 2.5. Determination of protein and data analysis

Protein was measured with the Lowry method, using bovine serum albumin as a standard. The agonist-induced $[Ca^{2+}]_i$ elevation is expressed as % increase over basal level. Protein kinase C activity is expressed as count per minute (cpm)/tube. Scatchard analysis was performed to obtain the dissociation constant ($K_d$) and the maximum binding site ($B_{max}$) in the receptor binding analysis. The results obtained are shown as means ± S.D. and statistical

difference of the values was determined by Student's *t*-test or analysis of variance (ANOVA).

### 2.6. Materials

Fetal bovine serum was obtained from Cell Culture Laboratory (Cleveland, OH, USA). Dulbecco's modified Eagle's medium (DMEM) and Eagle's minimum essential medium (EMEM) were obtained from Nissui Pharmaceutical (Tokyo, Japan). Fura 2 acetoxymethyl ester (Fura 2-AM) was from Dojindo (Kumamoto, Japan). Carbachol, atropine, phorbol 12-myristate 13-acetate (PMA) and LiCl were from Sigma (St. Louis, MO, USA). EGTA was from Nakarai Chemicals (Kyoto, Japan). Triton X-100 was from Wako (Tokyo, Japan). Collagenase was obtained from Worthington Biochemical (Freehold, NJ, USA). Protein kinase C enzyme assay system and [$^3$H]quinuclidinyl benzylate were obtained from Amersham (Buckinghamshire, UK). Other chemicals and drugs were of reagent grade or the highest quality available.

## 3. Results

### 3.1. Effect of PMA on carbachol-induced $[Ca^{2+}]_i$ elevation

Carbachol, a muscarinic receptor agonist, caused a transient increase in $[Ca^{2+}]_i$ with a peak at 10 s after its addition. The carbachol-induced $[Ca^{2+}]_i$ elevation occurred in a concentration-dependent manner with an $EC_{50}$ value of approximately 30 μM (data not shown). Treatment of the cells with 1 μM PMA for 2 min resulted in a reduction of the carbachol (100 μM)-induced $[Ca^{2+}]_i$ elevation (Fig. 1A). In contrast to short-term (2 min) treatment with PMA, carbachol caused a $[Ca^{2+}]_i$ elevation in the cells pretreated with 1 μM PMA for 48 h. In the cells treated with 1 μM PMA for 48 h, the carbachol-induced $[Ca^{2+}]_i$ elevation was not inhibited by further application of 1 μM PMA for 2 min (Fig. 1B). The results suggest that protein kinase C is down-regulated by treatment with 1 μM PMA for 48 h, and that carbachol elevates $[Ca^{2+}]_i$ in functionally protein kinase C down-regulated cells.

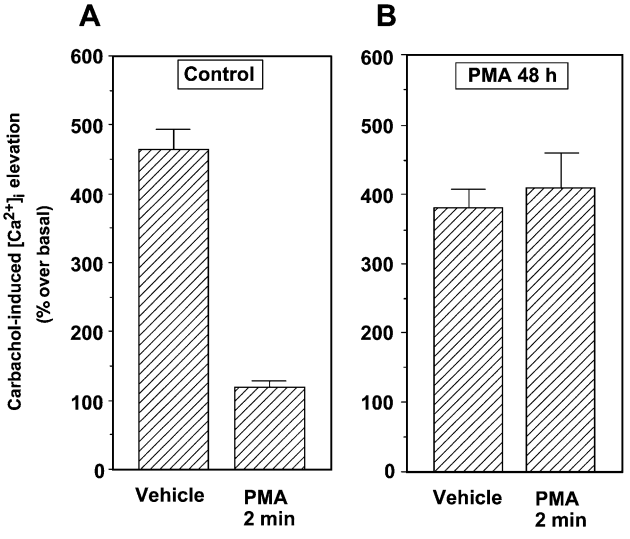


Fig. 1. Effect of PMA on carbachol-induced $[Ca^{2+}]_i$ elevation in 1321N1 human astrocytoma cells. (A) Short-term effect of PMA on carbachol-induced $[Ca^{2+}]_i$ elevation. PMA (1 μM) was added 2 min before addition of 100 μM carbachol. Results are expressed as % increase over basal level. Each column represents the mean ± S.D. of six experiments. Significant difference from the value of carbachol alone ( * $P<0.0001$, Student's *t*-test). (B) Long-term effect of PMA on carbachol-induced $[Ca^{2+}]_i$ elevation. PMA (1 μM) was added 2 min before addition of 100 μM carbachol in cells treated with 1 μM PMA for 48 h. Results are expressed as % increase over basal level. Each column represents the mean ± S.D. of six experiments.

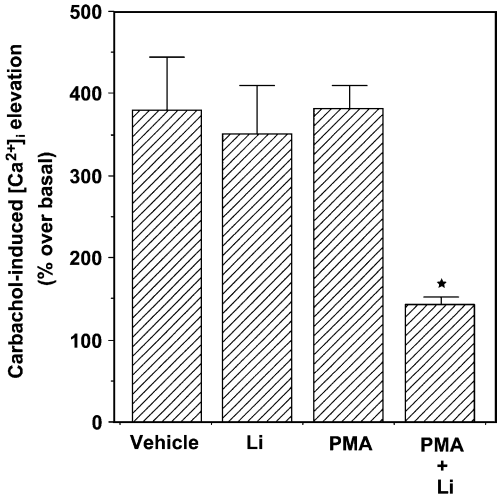


Fig. 2. Treatment of $Li^+$ and/or PMA for 48 h on carbachol-induced $[Ca^{2+}]_i$ elevation in 1321N1 human astrocytoma cells. Vehicle: treatment with vehicle for 48 h; Li: treatment with 1 mM LiCl for 48 h; PMA: treatment with 1 μM PMA for 48 h; Li + PMA: treatment with 1 μM PMA and 1 mM LiCl for 48 h. Carbachol-induced $[Ca^{2+}]_i$ elevation was measured in the cells after these treatments. Results are expressed as % increase over basal level. Each column represents the mean ± S.D. of six experiments. Carbachol-induced $[Ca^{2+}]_i$ elevation in cells treated with 1 μM PMA + 1 mM LiCl for 48 h was significantly lower than that in the cells under any other conditions ( * $P<0.05$, ANOVA post-hoc Scheffe).

### 3.2. Effect of $Li^+$ on carbachol-induced $[Ca^{2+}]_i$ elevation in protein kinase C down-regulated cells

The carbachol-induced $[Ca^{2+}]_i$ elevation was examined in cells treated with 1 mM $Li^+$ and/or 1 μM PMA for 48 h. Treatment of cells with 1 μM PMA or 1 mM $Li^+$ alone for 48 h did not change the carbachol-induced $[Ca^{2+}]_i$ elevation. The combined treatment of cells with 1 μM PMA and 1 mM $Li^+$ for 48 h caused a significant decrease in the carbachol-induced $[Ca^{2+}]_i$ elevation, compared with the elevation measured in cells treated with vehicle, 1 μM PMA or 1 mM $Li^+$ ($P<0.05$ ANOVA post-hoc Scheffe) (Fig. 2). Then, we examined the time course (0, 12, 24, 36, 48 h) of the $Li^+$ effect on the carbachol-induced $[Ca^{2+}]_i$

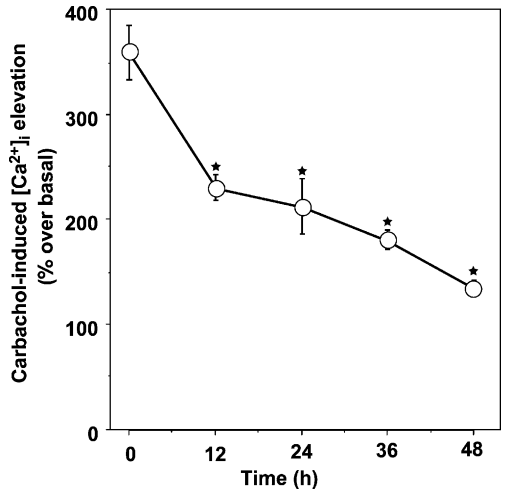


Fig. 3. Time course of the inhibitory effect of $Li^+$ on carbachol-induced $[Ca^{2+}]_i$ elevation in protein kinase C down-regulated 1321N1 human astrocytoma cells. Carbachol (100 μM)-induced $[Ca^{2+}]_i$ elevation was examined after pretreatment of cells with 1 μM PMA for 48 h combined with 1 mM LiCl for 0, 12, 24, 36 and 48 h. Each point represents the mean ± S.D. of six experiments. Carbachol (100 μM)-induced $[Ca^{2+}]_i$ elevation was significantly decreased by $Li^+$ treatment in a time-dependent manner (* $P<0.05$, ANOVA post-hoc Scheffe).

elevation in the protein kinase C down-regulated cells. $Li^+$ at 1 mM decreased the carbachol (100 μM)-induced $[Ca^{2+}]_i$ elevation in cells treated with 1 μM PMA for 48 h in a time-dependent manner (Fig. 3).

We also examined the concentration dependence of $Li^+$ in the inhibition of the carbachol-induced $[Ca^{2+}]_i$ elevation

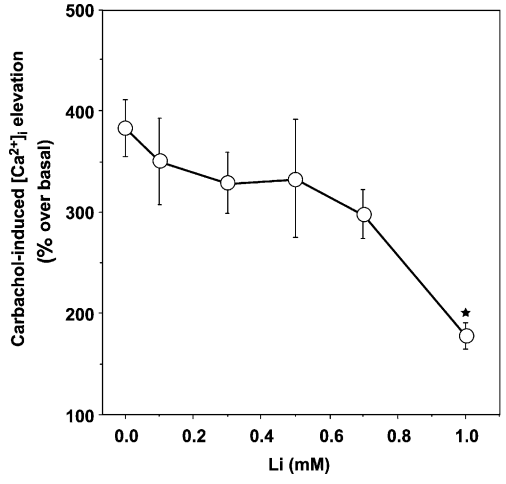


Fig. 4. Concentration dependence of the inhibitory effects of $Li^+$ on carbachol-induced $[Ca^{2+}]_i$ elevation in protein kinase C down-regulated 1321N1 human astrocytoma cells. Carbachol (100 μM)-induced $[Ca^{2+}]_i$ elevation was examined after pretreatment of cells with 1 μM PMA for 48 h combined with LiCl at the concentrations of 0, 0.1, 0.3, 0.5, 0.7 and 1.0 mM for 48 h. Each point represents the means ± S.D. of six experiments. $Li^+$ at the concentration of 1 mM significantly inhibited carbachol-induced $[Ca^{2+}]_i$ elevation compared with the effect of no $Li^+$ (* $P<0.05$, ANOVA post-hoc Scheffe).

in protein kinase C down-regulated cells. The cells were pretreated with $Li^+$ for 48 h in a concentration range of 0.1 to 1 mM. $Li^+$ tended to decrease the carbachol (100 μM)-induced $[Ca^{2+}]_i$ elevation in a concentration-dependent manner and $Li^+$ at 1 mM inhibited significantly the carbachol-induced $[Ca^{2+}]_i$ elevation under the protein kinase C down-regulated condition (Fig. 4).

### *3.3. Effect of Li+ on protein kinase C activity in protein kinase C down-regulated cells*

Protein kinase C activity was examined in cells pretreated with 1 mM $Li^+$ and/or 1 μM PMA for 48 h (Fig. 5). Protein kinase C translocated from the cytosolic to the membrane fraction after incubation with 1 μM PMA for 30 min in cells pretreated with vehicle or 1 mM $Li^+$ for 48 h, suggesting that 1 mM $Li^+$ does not affect protein kinase C activity. The cells treated with 1 μM PMA for 48 h had a lower protein kinase C activity in both the membrane and

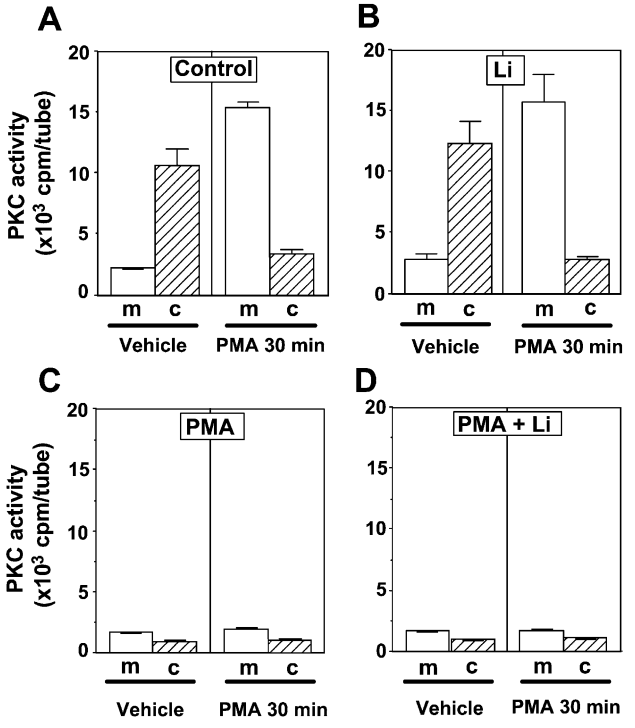


Fig. 5. Effects of $Li^+$ and/or PMA treatment for 48 h on protein kinase C activity in 1321N1 human astrocytoma cells. The cells were pretreated with vehicle as control (A), 1 mM LiCl (B), 1 μM PMA (C), or 1 μM PMA + 1 mM LiCl (D) for 48 h. Then, the cells were stimulated with vehicle (none) or 1 μM PMA for 30 min (PMA 30 min). Membrane (m; open column) and cytosol (c; hatched column) fractions were obtained, and protein kinase C activity was measured in both membrane and cytosol fractions as described in Methods. Each column represents the mean ± S.D. of three experiments. Protein kinase C translocated from the cytosol to the membrane fraction after incubation with 1 μM PMA for 30 min in the cells treated with vehicle (A) or 1 mM LiCl (B) for 48 h. However, the cells treated with 1 μM PMA for 48 h showed low protein kinase C activity in both membrane and cytosol fractions (C), and 1 mM $Li^+$ failed to restore the low protein kinase C activity (D).

cytosolic fractions than did the cells treated with vehicle, reflecting the down-regulation of protein kinase C. Treatment with 1 mM $Li^+$ for 48 h did not restore the low protein kinase C activity in the cells treated with 1 μM PMA for 48 h.

### *3.4. Effect of Li+ on [$^3$H]quinuclidinyl benzylate binding in protein kinase C down-regulated cells*

[$^3$H]Quinuclidinyl benzylate, a muscarinic receptor antagonist, specifically bound to membranes derived from 1321N1 cells. Scatchard analysis of [$^3$H]quinuclidinyl benzylate binding revealed a single binding site in membranes derived from the cells (Fig. 6). Treatment of the cells with 1 μM PMA for 48 h did not affect the $K_d$ value or $B_{max}$ value of [$^3$H]quinuclidinyl benzylate binding, although it down-regulated protein kinase C (Fig. 6, Table 1). Furthermore, treatment of the cells with $Li^+$ for 48 h did not affect the $K_d$ value or $B_{max}$ value of [$^3$H]quinuclidinyl benzylate binding in either normal or protein kinase C down-regulated cells (Fig. 6, Table 1). The results indicate that 1 mM $Li^+$ does not change the affinity or number of muscarinic receptors itself, although it affects the signal transduction in the protein kinase C down-regulated condition.

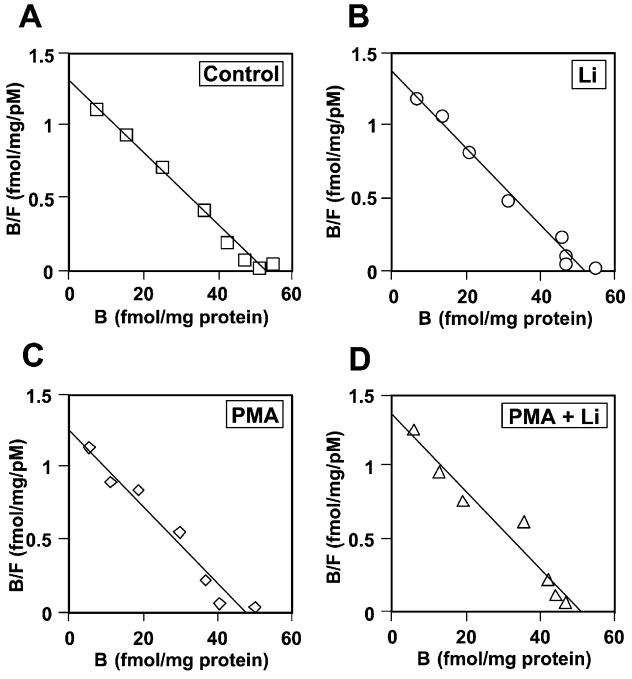


Fig. 6. Scatchard analysis of [$^3$H]quinuclidinyl benzylate binding to membranes derived from 1321N1 human astrocytoma cells. The cells were pretreated with vehicle as control (A), 1 mM LiCl (B), 1 μM PMA (C) or 1 μM PMA + 1 mM LiCl (D) for 48 h. The membranes were prepared as described in Methods in detail. Membranes (250 μg) were incubated with [$^3$H]quinuclidinyl benzylate (0.01 to 2.0 nM) with or without 1 μM atropine. The data are presented as a Scatchard plot. Each point represents the mean value from triplicate assays and the data are representative of three similar experiments.

Table 1
$K_d$ and $B_{max}$ of [$^3$H]quinuclidinyl benzylate binding to membranes of 1321N1 human astrocytoma cells

| Treatment | $K_d$ (pM) | $B_{max}$ (fmol/mg protein) |
|---|---|---|
| Control | 48.0 ± 3.9 | 50.7 ± 2.1 |
| Li 1 mM, 48 h | 47.1 ± 2.9 | 56.5 ± 2.8 |
| PMA 1 μM, 48 h | 50.6 ± 1.5 | 49.0 ± 3.4 |
| Li 1 mM + PMA 1 μMm, 48 h | 49.0 ± 1.4 | 53.7 ± 4.7 |

The $K_d$ and $B_{max}$ of [$^3$H]quinuclidinyl benzylate binding to membranes were not significantly different among these groups ($P < 0.05$, ANOVA post-hoc Scheffe) ($n = 3$, mean ± S.D.).

## 4. Discussion

Treatment of cells with PMA for 2 min resulted in a reduction of the carbachol-induced $[Ca^{2+}]_i$ elevation, consistent with other reports (Orellana et al., 1985; Yang et al., 1994). However, PMA did not affect the carbachol-induced $[Ca^{2+}]_i$ elevation after the cells were treated with PMA for 48 h (Kurita et al., 2000), indicating that the low protein kinase C activity was due to the down-regulation of protein kinase C in cells. Thus, it is assumed that the carbachol-induced $[Ca^{2+}]_i$ elevation is regulated by protein kinase C, and that the regulation disappears in functionally protein kinase C-deficient cells.

The suitable concentration of $Li^+$ in serum for the therapy of bipolar mood disorder is reported to be 0.5–1.0 mM (= mEq/l) (Atack et al., 1995). When the serum concentration of $Li^+$ is higher than 2 mM in patients, toxicity appears clinically, such as nausea, fine tremors, diarrhea, ataxia confusion and slurred speech (Atack et al., 1995). There are several reports concerning the mechanism of $Li^+$ action, such as attenuation of G protein function (Avissar et al., 1988; Drummond, 1988), down-regulation of protein kinase Cα (Bitran et al., 1995) and inhibition of inositol monophosphatase (Moscovich et al., 1990). It has been shown that $Li^+$ at a therapeutic concentration has no effect on the amount of $IP_3$ under normal or inositol-rich conditions (Dixon et al., 1992), although it has been reported to inhibit potently an intracellular enzyme, inositol monophosphatase, under conditions in which there is depletion of free inositol (Varney et al., 1992). Therefore, it is unclear whether $Li^+$ at a therapeutic concentration inhibits inositol monophosphatase sufficiently under inositol-rich conditions. However, the repeated administration of $Li^+$ for several weeks is necessary for its pharmacological effect in vivo (Atack et al., 1995).

Treatment of 1321N1 cells with 1 mM $Li^+$, a therapeutic concentration, in inositol-rich medium for 48 h did not change the carbachol-induced $[Ca^{2+}]_i$ elevation in our experiments. Thus, it is suggested that 1 mM $Li^+$ alone is

without effect on the carbachol-induced $[Ca^{2+}]_i$ elevations. However, in protein kinase C down-regulated cells 1 mM $Li^+$ significantly and potently inhibited the carbachol-induced $[Ca^{2+}]_i$ elevation even when the experiments were performed under inositol-rich conditions. This inhibitory effect of $Li^+$ was time (12–48 h)- and concentration (0.1–1.0 mM)-dependent under the protein kinase C down-regulated condition. From these results, it is assumed that treatment of the cells with a therapeutic concentration of $Li^+$ for a certain period results in a reduction of agonist-induced intracellular $Ca^{2+}$ mobilization only when protein kinase C is down-regulated. To our knowledge, this is the first demonstration that $Li^+$ at a therapeutic concentration affects agonist-induced $[Ca^{2+}]_i$ elevation in protein kinase C down-regulated cells under inositol-rich conditions.

Studies of chronic $Li^+$ administration in rats have demonstrated a reduction in membrane-associated protein kinase Cα and ε in the subiculum and in CA1 regions of the hippocampus (Manji et al., 1993). The reduction was observed only after 4 weeks' treatment with $Li^+$, and not after a 5-day treatment. Thus, it is thought that $Li^+$ decreases protein kinase C activity after long-term treatment several weeks. In spite of the report of protein kinase C inhibition by $Li^+$, the present study demonstrated that the treatment of cells with $Li^+$ at a therapeutic concentration for 2 days never affected protein kinase C activity, suggesting that 2 days may not be long enough to inhibit protein kinase C activity. Nevertheless, the 2-day treatment with $Li^+$ clearly reduced the agonist-induced $[Ca^{2+}]_i$ elevation under protein kinase C down-regulated conditions. Thus, it is assumed that $Li^+$ has an action other than the inhibition of protein kinase C to attenuate the agonist-induced $[Ca^{2+}]_i$ elevation. A protein kinase C down-regulated condition is important for the inhibitory effect of $Li^+$ on $[Ca^{2+}]_i$.

When protein kinase C is down-regulated in 1321N1 cells, it is possible that the affinity or number of muscarinic receptors is changed. However, Scatchard analysis of [$^3$H]quinuclidinyl benzylate binding demonstrated that neither the affinity nor the number of muscarinic receptors was changed by $Li^+$ treatment. Thus, $Li^+$ is able to inhibit agonist-induced $[Ca^{2+}]_i$ elevation under protein kinase C-deficient conditions without a change in receptor function. The present finding that $Li^+$ is effective under protein kinase C-deficient conditions is potentially important for establishing effective conditions for $Li^+$ therapy in patients with bipolar disorder. However, further studies are necessary to delineate the detailed mechanism of $Li^+$ by examining the effect of $Li^+$ on inositol monophosphatase, phosphatidylinositol turnover, Gq protein function and the function of regulators of G protein signaling under protein kinase C down-regulated conditions.

In conclusion, the present study demonstrates that $Li^+$ at a therapeutic concentration appears to exert its inhibitory effect on agonist-induced $[Ca^{2+}]_i$ elevation when protein kinase C activity is down-regulated without affecting protein kinase C activity or receptor function.

## References


Allison, J.H., Blister, M.E., 1976. Inhibition of the effect of lithium on brain inositol by atropine and scopolamine. Biochem. Biophys. Res. Commun. 68, 1332–1338.

Atack, J.R., Rapoport, S.I., Varley, C.L., 1993. Characterization of inositol monophosphatase in human cerebrospinal fluid. Brain Res. 613, 305–308.

Atack, J.R., Brought, H.B., Pollack, S.J., 1995. Inositol monophosphatase—a putative target for $Li^+$ in the treatment of bipolar disorder. Trends Neurosci. 18, 343–349.

Avissar, S., Schreiber, G., Danon, A., Belmaker, R.H., 1988. Lithium inhibits adrenergic and cholinergic increases in GTP binding in rat cortex. Nature 331, 440–442.

Bebchuk, J.M., Arfken, C.L., Dolan-Manji, S., Murphy, J., Hasanat, K., Manji, H.K., 2000. A preliminary investigation of a protein kinase C inhibitor in the treatment of acute mania. Arch. Gen. Psychiatry 57, 95–97.

Berridge, M.J., Irvine, R.F., 1984. Inositol trisphosphate, a novel second messenger in cellular signal transduction. Nature 312, 315–321.

Bitran, J.A., Manji, H.K., Potter, W.Z., Gusovsky, F., 1995. Down-regulation of PKC alpha by lithium in vitro. Psychopharmacol. Bull. 31, 449–452.

Dixon, J.F., Lee, C.H., Los, G.V., Hokin, L.E., 1992. Lithium enhances accumulation of [$^3$H]inositol radioactivity and mass of second messenger inositol 1,4,5-trisphosphate in monkey cerebral cortex slices. J. Neurochem. 59, 2332–2335.

Drummond, A.H., 1988. Lithium affects G-protein receptor coupling. Nature 331, 388.

Friedman, E., Hoau Yan, W., Levinson, D., Connell, T.A., Singh, H., 1993. Altered platelet protein kinase C activity in bipolar affective disorder, manic episode. Biol. Psychiatry 33, 520–525.

Kurita, M., Mashiko, H., Ogata, M., Kumasaka, T., Niwa, S., Nakahata, N., Takano, S., Nakanishi, H., 2000. Discrimination of histamine $H_1$ and muscarinic receptor-mediated signalling pathways by phorbol ester in human astrocytoma cells. Clin. Exp. Pharmacol. 27, 208–211.

Manji, H.K., Etcheberrigaray, R., Chen, G., Olds, J.L., 1993. Lithium decreases membrane-associated protein kinase C in hippocampus: selectivity for the alpha isozyme. J. Neurochem. 61, 2303–2310.

Moscovich, D.G., Belmaker, R.H., Agam, G., Livne, A., 1990. Inositol-1-phosphatase in red blood cells of manic-depressive patients before and during treatment with lithium. Biol. Psychiatry 27, 552–555.

Nishizuka, Y., 1986. Studies and perspectives of protein kinase C. Science 233, 305–312.

Orellana, S.A., Solski, P.A., Brown, J.H., 1985. Phorbol ester inhibits phosphoinositide hydrolysis and calcium mobilization in cultured astrocytoma cells. J. Biol. Chem. 260, 5236–5239.

Schou, M., 1997. Forty years of lithium treatment. Arch. Gen. Psychiatry 54, 9–13.

Streb, H., Irvine, R.F., Berridge, M.J., Schulz, I., 1983. Release of $Ca^{2+}$ from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. Nature 306, 67–69.

Varney, M.A., Godfrey, P.P., Drummond, A.H., Watson, S.P., 1992. Chronic lithium treatment inhibits basal and agonist-stimulated responses in rat cerebral cortex and GH3 pituitary cells. Mol. Pharmacol. 42, 671–678.

Yang, C.M., Sung, T.C., Ong, R., Hsieh, J.T., Luo, S.F., 1994. Effect of phorbol ester on phosphoinositide hydrolysis and calcium mobilization in cultured canine tracheal smooth muscle cells. Naunyn-Schmiedeberg's Arch. Pharmacol. 350, 77–83.