The Inhibitory Effect of Li⁺ on Contractile Elements of Intestinal Smooth Muscle

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ABSTRACT—The mechanism of the inhibitory effect of Li⁺ on contraction was examined in guinea pig ileal longitudinal smooth muscle. Li⁺-substitution (68.4 mM) reversed contractions induced by high K⁺ (45.4 mM), carbachol (1 μM) and histamine (1 μM) without changing the cytosolic Ca²⁺ level. Li⁺ also had no effect on the increase in ⁴⁰Ca²⁺ uptake stimulated by high K⁺. High K⁺ transiently increased myosin light chain (MLC) phosphorylation, reaching a peak at 6–9 sec. Li⁺-substitution inhibited the high K⁺-induced MLC phosphorylation. In permeabilized ileal strips, contraction induced by 1 μM Ca²⁺ was inhibited by 10 mM Li⁺. The inhibitory effect was antagonized by increasing the concentration of Ca²⁺ or calmodulin. In the permeabilized muscle in which MLC was previously thiphosphorylated with 1 mM ATP/S and 3 μM Ca²⁺, ATP induced contraction in Ca²⁺ free buffer. Li⁺ added during this contraction did not show an inhibitory effect. In contrast, when 30 mM Li⁺ was added during the thiphosphorylation, the contraction induced by the subsequent addition of ATP was inhibited. Li⁺ (30 mM) changed neither the rate of relaxation induced by removing external Ca²⁺ in permeabilized muscle nor the rate of dephosphorylation of MLC induced by crude phosphatase extracted from the ileum. Li⁺ (15 mM), on the other hand, inhibited the rate of phosphorylation of MLC caused by crude MLC kinase extracted from the ileum. Li⁺ did not inhibit the calmodulin activity as measured with the (Ca²⁺ + Mg²⁺)-ATPase activity of the erythrocyte membrane. These results suggest that the inhibitory effect of Li⁺ on contractions is attributable to the inhibition of MLC kinase in guinea pig ileum.

Keywords: Li⁺ (lithium ion), Smooth muscle (intestine), Contractile element, Myosin light chain kinase, Cytosolic Ca²⁺ level

Li⁺ has been shown to have various biological actions such as inhibition of phosphatidylinositol turnover (1, 2) and adenylate cyclase (3, 4). In intestinal smooth muscle, Li⁺ relaxes contractions induced by high concentrations of KCl and receptor agonists (5–7). Treatment of guinea pig ileal muscle with 68.4 mM Li⁺ solution increased the cellular Li⁺ content to 10–20 mmol/kg wet wt. in 30–60 min (8) and the inhibitory effect increased with the accumulation of Li⁺ in the cell (9–11). Recently, we have reported that Li⁺-induced relaxation is not due to a decrease in cytosolic Ca²⁺ level ([Ca²⁺]) and that Li⁺ relaxes Ca²⁺-induced contraction in permeabilized tissue (11). The purpose of the present experiments is to further clarify the mechanism for the inhibitory effect of Li⁺ in guinea pig ileal smooth muscle.

MATERIALS AND METHODS

Preparations and solutions

Ileum was isolated from male guinea pigs (300–400 g) after stunning by a sharp blow on the neck and exsanguination. Longitudinal smooth muscle strips, approximately 5-mm-wide and 10-mm-long, were prepared. Normal physiological salt solution (PSS) contained: 136.9 mM NaCl, 5.4 mM KCl, 1.5 mM CaCl₂, 1.0 mM MgCl₂, 23.8 mM NaHCO₃ and 5.5 mM glucose. High K⁺ solutions were made by hyperosmotically adding 45.4 mM or 68.4 mM KCl to PSS unless otherwise stated. These solutions were saturated with a 95% O₂ and 5% CO₂ mixture at 37°C.
Simultaneous measurements of muscle tension and [Ca\(^{2+}\)]

Muscle strips were treated with PSS containing 5 \(\mu\)M acetoxyethyl ester of fura-2 and 0.03% cremophor EL for 6 hr at room temperature. The muscle strips were illuminated alternately (48 Hz) at excitation wavelengths of 340 nm and 380 nm, and the intensity of the fluorescence at 500 nm was measured with a fluorimeter (CAF-100; Japan Spectroscopic, Tokyo). The ratio of the fluorescence due to excitation at 340 nm to that at 380 nm (R340/380) was used as an indicator of relative [Ca\(^{2+}\)]. Absolute [Ca\(^{2+}\)] was not calculated because the dissociation constant of fura-2 for Ca\(^{2+}\) might be different from that obtained in vitro (12, 13). Quantitative comparison of the [Ca\(^{2+}\)] was made by taking resting and high K\(^{+}\)-stimulated [Ca\(^{2+}\)] as 0 and 100%, respectively, as previously described (14, 15). Muscle tension was recorded isometrically with a force displacement transducer. Each muscle strip was attached to a holder under a resting tension of 10 mN. Because hyperosmotic solution interferes with fura-2 fluorescence (16), high K\(^{+}\)-solution was made by substituting 45.4 mM NaCl of the PSS with equimolar KCl.

\(^{45}\)Ca\(^{2+}\) uptake

\(^{45}\)Ca\(^{2+}\) uptake was measured as described by Karaki and Weiss (17). The muscle strips were incubated in the PSS containing \(^{45}\)Ca\(^{2+}\) (2.0 mCi/ml) for 6 min. In some preparations, muscle strips were pretreated with PSS containing 68.4 mM Li\(^{+}\) for 40 min. The \(^{45}\)Ca\(^{2+}\) incubation was followed by washing for 30 min with ice-cold La\(^{3+}\) solution containing 73.8 mM LaCl\(_3\), 5.5 mM glucose and 24 mM Tris-HCl adjusted to pH 6.9 at 0°C. \(^{45}\)Ca\(^{2+}\) was extracted from the muscle strips overnight with a solution containing 2 mM ethyleneglycol bis (\(\beta\)-aminoethyl ether) tetraacetic acid (EGTA). A 1-ml aliquot of scintillation mixture (ACS II; Amersham Japan, Tokyo) was added to the extract, and the radioactivity was counted with a liquid scintillation spectrometer (LSC-3500; AlokA, Mitaka).

Permeabilized muscle

Permeabilized muscle strips, 0.2 mm in width and 3 mm in length, were made by treating the muscle with Triton X-100 as described by Sparrow et al. (18). Each muscle strip was attached to a holder under a resting tension of 0.5 mN at 24°C and muscle tension was recorded isometrically. The relaxing solution containing: 50 mM KCl, 20 mM imidazole, 2 mM EGTA, 4 mM MgCl\(_2\), 2 mM Na\(_2\)-ATP, 3 mM KH\(_2\)PO\(_4\), 3 mM K\(_2\)HPO\(_4\), 1 mM Na\(_3\)N\(_4\) and 0.1 \(\mu\)M calmodulin. The Ca\(^{2+}\) solution was made by adding appropriate amounts of CaCl\(_2\) to the relaxing solution. The pH of the relaxing solution and Ca\(^{2+}\) solution were adjusted to 6.8 at 24°C. The apparent binding constant used for the Ca\(^{2+}\)-EGTA complex was assumed to be 10\(^6\) M\(^{-1}\) (19). The Li\(^{+}\) solution was made by substituting 10 mM KCl of the relaxing solution and Ca\(^{2+}\) solution with equimolar LiCl.

(Ca\(^{2+}\)+Mg\(^{2+}\))-ATPase activity of the erythrocyte membrane

The effect of Li\(^{+}\) on calmodulin activity was examined using the (Ca\(^{2+}\)+Mg\(^{2+}\))-ATPase activity of erythrocyte membranes as described by Gopinath and Vincenzi (20). Erythrocyte membrane ghosts were prepared by the method of Ozaki et al. (21). The (Ca\(^{2+}\)+Mg\(^{2+}\))-ATPase assay was performed at 25°C in a medium containing: 100 mM NaCl, 10 mM KCl, 3 mM MgCl\(_2\), 20 mM Tris-maleate (pH 6.8), 0.1 mM ouabain, 30 mM calmodulin, erythrocyte membrane ghosts (1 mg protein/ml) and appropriate amounts of CaCl\(_2\). The reaction was started by the addition of 2 mM ATP to the medium and stopped by the addition of 10% trichloroacetic acid to the medium. The Li\(^{+}\) solution was made by substituting NaCl of the medium with equimolar LiCl. The amount of inorganic phosphate liberated during the 20-min incubation period was determined as described by Martin and Doty (22).

Myosin light chain (MLC) phosphorylation

Muscle strips were frozen in acetone-dry ice and crushed in liquid nitrogen. The crushed powder was mixed with 10 volumes of urea-buffer containing: 8.5 M urea, 40 mM dithiothreitol, 2% Nonidet P-40, 1% sodium-dodecyl sulfate (SDS) and 2% carrier ampholyte for isoelectric focusing (mixture of 4 volumes of pH 4 - 6.5 carrier ampholyte and 1 volume of pH 3 - 10 carrier ampholyte). The sample was then centrifuged for 20 min at 10,000 \(\times\) g, and the supernatant was collected. MLC phosphorylation was measured by two dimensional electrophoresis (isolectric-SDS polyacrylamide gel electrophoresis) as described by O'Farrel (23) using a Fast System (Pharmacia Fine Chemical, Uppsala, Sweden). Proteins were visualized by silver staining and quantified by scanning densitometry (CS-9300PC; Shimadzu Co., Kyoto).

MLC kinase and phosphatase activities

Guinea pig ileal muscle strips (2 g) were frozen and crushed in liquid nitrogen. The crushed powder was mixed with 2 ml Buffer-A containing: 20 mM Tris-HCl (pH 7.0), 600 mM KCl, 2 mM EGTA, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 1 mg/ml leupeptin and 1 mg/ml trypsin inhibitor. The sample was then centrifuged for 30 min at 80,000 \(\times\) g, and the supernatant was collected. The protein concentration of the extract was measured by the Bradford method (24) and diluted to at 5 mg/ml. A crude MLC kinase preparation was prepared by inhibiting serine/threonine-phosphatases (types 1 and
2A) of the extract with 300 nM calyculin A (25). A crude phosphatase preparation was made by inhibiting the kinase activity of the extract with 30 μM K252a, a non-selective kinase inhibitor (26, 27).

The activity of the crude MLC kinase preparation was measured at 25°C in a medium containing: 20 mM Tris-HCl (pH 7.0), 2 mM MgCl₂, 2 mM EGTA, 60 mM KCl, 0.2 mg/ml MLC, 100 ng/ml calmodulin, Ca²⁺-EGTA buffer (pCa 5) and the crude MLC kinase sample (0.5 mg/ml). The reaction of MLC kinase was started by adding 0.5 mM ATP with 10 μCi γ-[³²P]ATP to the medium and stopped by adding 2 ml washing solution (5% trichloroacetic acid and 1% Na₄P₂O₇). The reaction medium was removed from the precipitated proteins by 3 washings using a small column with filter, and the collected proteins with washing solution heated to 80°C for 10 min to remove nonspecific binding of γ-[³²P]ATP, and then washed 3 times again. The radioactivity of the reaction medium was determined by liquid scintillation spectrometer (Beckman LS180; Fullerton, CA, USA).

The measurement of the phosphatase activity was performed as follows: Purified MLC was phosphorylated previously by adding 0.5 mM ATP with γ-[³²P]ATP in a medium containing: 20 mM Tris-HCl (pH 7.0), 2 mM MgCl₂, 2 mM EGTA, 60 mM KCl, 0.2 mg/ml MLC, 1 μg/ml calmodulin, Ca²⁺-EGTA buffer (pCa 5) and 10 μg/ml MLC kinase purified from chicken gizzard. Six minutes after the addition of ATP, nonselective kinase inhibitor (30 μM K252a) and 30 mM KCl or LiCl were added, and the crude phosphatase preparation (0.5 mg/ml) was added 1 min later. Measurement of MLC phosphorylation was started from this point. The amount of phosphorylated MLC was not changed by adding K252a and KCl or LiCl without the crude phosphatase sample to the medium, suggesting that there were no contaminating phosphatases in the medium.

**Chemicals**

Chemicals used were carbachol and histamine dihydrochloride (Wako Pure Chemicals, Tokyo); trifluoperazine, ouabain, verapamil, phenylmethylsulfonyl fluoride, trypsin inhibitor, ATP and Triton X-100 (Sigma Chemical Co., St. Louis, MO, USA); cremophor EL, dithiothreitol and Nonidet P-40 (Nacalai Tesque, Kyoto); W-7 (N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride; Seikagaku Kogyo Co., Tokyo); K252a (Kyowa Medex, Tokyo); acetoxymethyl ester of fura-2 (Dojindo Laboratories, Kumamoto); ATPγS (Boehringer Mannheim, Mannheim, United Germany); and carrier ampholyte (Pharmacia Fine Chemical). Calmodulin was purified from swine testis according to the method of Walsh (28). Calyculin A, MLC purified from chicken gizzard and MLC kinase purified from chicken gizzard and MLC kinase purified from chicken gizzard were purchased from Seikagaku Kogyo Co., Tokyo.

**Fig. 1.** Effects of Li⁺ on muscle tension and [Ca²⁺], stimulated by KCl (A), carbachol (B) and histamine (C). The concentrations used were as follows: Li⁺, 68.4 mM; KCl, 45.4 mM; carbachol, 1 μM; histamine, 1 μM; and EGTA, 4 mM. Relative [Ca²⁺], was measured by R340/380. 100% represents the high K⁺-induced sustained [Ca²⁺]. Li⁺ was added after the high K⁺ or receptor agonist-induced [Ca²⁺], and the tension reached a steady state. In panel B, Li⁺ was removed to investigate the reversibility of Li⁺-induced relaxation. EGTA was sequentially added. The trace represents typical results from one out of 4–6 experiments.
chicken gizzard were donated by Dr. N. Fusetani (The University of Tokyo), Dr. M. Ito (The University of Mie) and Dr. T. Kanoh (The University of Mie), respectively.

**Statistics**

The results of the experiments are expressed as means±S.E.M. Student’s t-test was used for statistical analyses of the results.

**RESULTS**

**Cytosolic Ca$^{2+}$ level and $^{45}$Ca$^{2+}$ uptake**

High K$^+$ (45.4 mM), 1 μM carbachol and 1 μM histamine increased [Ca$^{2+}$], and muscle tension in guinea pig ileal muscle strips. Li$^+$ (68.4 mM), added to muscle contracted by one of these stimulants, decreased muscle tension without changing [Ca$^{2+}$] (Fig. 1).

High K$^+$ (45.4 mM) increased $^{45}$Ca$^{2+}$ uptake from 198.2±7.96 nmol/mg wet wt./6 min to 300.9±18.2 nmol/mg wet wt./6 min. A Ca$^{2+}$ channel blocker, 10 μM verapamil, decreased the high K$^+$-stimulated $^{45}$Ca$^{2+}$ uptake to the resting level (218.9±7.58 nmol/mg wet wt./6 min). Treatment of the muscle with 68.4 mM Li$^+$ for 40 min did not inhibit $^{45}$Ca$^{2+}$ uptake stimulated by high K$^+$ (320.6±4.17 nmol/mg wet wt./6 min) (Fig. 2).

**MLC phosphorylation**

Figure 3 shows the effect of Li$^+$ on the high K$^+$-induced increase in MLC phosphorylation. In unstimulated muscle, phosphorylated MLC was 14.1±0.47% of the total MLC. Phosphorylated MLC increased to 30.1±2.95% at 6–9 sec and then decreased to 22.4±1.8% at 3 min after the addition of high K$^+$. The muscle contraction also reached the maximum at 6–9 sec and then decreased to 88.4±3.26% of the maximum at 3 min. In the muscle pretreated with 68.4 mM Li$^+$ for 40 min, high K$^+$-induced contraction was inhibited to 38.6±2.59% at 6–9 sec and 18.5±3.18% at 3 min of the maximum level in the absence of Li$^+$. The amount of phosphorylated MLC was also inhibited to 11.1±1.10% at 6–9 sec and 13.2±1.80% at 3 min of the total MLC.

**Permeabilized muscle**

In permeabilized muscle, 1 μM Ca$^{2+}$ caused sustained contraction. After the contraction reached a steady level, 10 mM Li$^+$, 30 μM trifluoperazine or 60 μM W-7 was added. Each of these substances produced a reduction in the Ca$^{2+}$-induced contraction by approximately 50% (Li$^+$, 42.9±8.20%; TFP, 56.0±4.37%; and W-7, 46.4±4.1 %). The tension reduction by these inhibitors was reversed by the addition of 0.4 μM calmodulin or 3 μM Ca$^{2+}$ (Fig. 4). A nonselective kinase inhibitor, 0.3 μM K252a, also produced a reduction of the Ca$^{2+}$-induced contraction by 75.14±3.14%. However, the inhibitory effect of K252a was not reversed by the addition of calmodulin or Ca$^{2+}$ (Fig. 4).

In permeabilized muscle, it was shown that the addition of 1 mM ATP$_7$S and 3 μM Ca$^{2+}$ (without ATP) thiophosphorylates MLC. After the thiophosphorylation of MLC,
2 mM ATP caused a sustained contraction in the absence of Ca\(^{2+}\) (64.2 ± 7.14% of 10 μM Ca\(^{2+}\)-induced contraction at the peak and 43.3 ± 6.67% at the sustained level measured at 15 min) as shown in Fig. 5 (B and D). Addition of 30 mM Li\(^+\) did not inhibit this contraction (Fig. 5A). When 30 mM Li\(^+\) was added during the thiophosphorylation (Fig. 5C), however, the ATP-induced contraction was inhibited to 43.5 ± 1.90% at the peak and to 21.0 ± 3.17% at 15 min of the 10 μM Ca\(^{2+}\)-induced contraction. These results are summarized in Fig. 5D.

We further examined the effect of Li\(^+\) on the rate of relaxation due to Ca\(^{2+}\)-removal in permeabilized muscle precontracted with 3 μM Ca\(^{2+}\). Li\(^+\) (30 mM) did not change the rate of relaxation of muscle tension due to Ca\(^{2+}\)-removal (n=4, data not shown).

**MLC kinase and phosphatase activity**

Figure 6 shows the effect of Li\(^+\) on the activities of crude MLC kinase and crude phosphatase. Crude MLC kinase phosphorylated MLC in a time-dependent manner. Although 5 mM Li\(^+\) was ineffective, 15 mM Li\(^+\) significantly decreased the rate of MLC phosphorylation (Fig. 6A). As shown in Fig. 6B, 30 mM Li\(^+\) had no effect on the rate of dephosphorylation of MLC induced by the crude phosphatase preparation.
Fig. 6. Effects of Li⁺ on MLC kinase (MLCK) activity (A) and phosphatase activity (B). MLC kinase activity and phosphatase activity are shown by mol Pi/mol MLC. A: open circle: control (KCl, 30 mM), closed circle: 5 mM LiCl and 25 mM KCl, open triangle: 15 mM LiCl and 15 mM KCl. B: open circle: Buffer-A (see method), closed circle: crude phosphatase preparation (0.5 mg/ml) prepared from guinea pig ileum (control), open triangle: crude phosphatase preparation pretreated with LiCl (30 mM). Trace of typical results, one of three experiments.

Fig. 7. Effects of Li⁺, trifluoperazine (TFP) and W-7 on (Ca²⁺ + Mg²⁺)-ATPase activity of the erythrocyte membrane. 100% represents the (Ca²⁺ + Mg²⁺)-ATPase activity stimulated by 2 mM ATP in the presence of 10 μM Ca²⁺ and 30 nM calmodulin. Each point represents the mean of 4–6 experiments, and S.E.M. is shown by a vertical bar.

**High K⁺ and Li⁺ effects**

High K⁺, carbachol and histamine induced sustained increases in [Ca²⁺], and muscle tension in the guinea pig ileum, and Li⁺ relaxed these contractions without changing [Ca²⁺]. Consistent with this observation, Li⁺ did not change the 45Ca²⁺ uptake stimulated by high K⁺. It has been demonstrated that Li⁺ does not change Ca²⁺ efflux in rat uterus (6, 7). These results suggest that Li⁺ inhibits smooth muscle contraction without changing Ca²⁺ movements across the plasma membrane. In contrast to the results obtained in the guinea pig ileum, it has been reported that cytosolic Li⁺ inhibited 45Ca²⁺ accumulation in the guinea pig taenia coli (9). The reason for this discrepancy has yet to be clarified.

**45Ca²⁺ uptake**

As shown in Fig. 7, calmodulin inhibitors, 10–300 μM trifluoperazine and 10 μM–3 mM W-7, inhibited the Ca²⁺ and calmodulin dependent (Ca²⁺ + Mg²⁺)-ATPase activity of erythrocyte membranes in a dose dependent manner. However, Li⁺ (1–100 mM) had no effect on the (Ca²⁺ + Mg²⁺)-ATPase activity. Li⁺ (30 mM) did not inhibit the (Ca²⁺ + Mg²⁺)-ATPase activity stimulated by Ca²⁺ (0.1–100 μM) and calmodulin remaining in the membrane preparation (n=4, data not shown).

**Calmodulin activity**

As shown in Fig. 6, calmodulin inhibitors, 10–300 μM trifluoperazine and 10 μM–3 mM W-7, inhibited the Ca²⁺ and calmodulin dependent (Ca²⁺ + Mg²⁺)-ATPase activity of erythrocyte membranes in a dose dependent manner. However, Li⁺ (1–100 mM) had no effect on the (Ca²⁺ + Mg²⁺)-ATPase activity. Li⁺ (30 mM) did not inhibit the (Ca²⁺ + Mg²⁺)-ATPase activity stimulated by Ca²⁺ (0.1–100 μM) and calmodulin remaining in the membrane preparation (n=4, data not shown).

**DISCUSSION**

High K⁺, carbachol and histamine induced sustained increases in [Ca²⁺], and muscle tension in the guinea pig ileum, and Li⁺ relaxed these contractions without changing [Ca²⁺]. Consistent with this observation, Li⁺ did not change the 45Ca²⁺ uptake stimulated by high K⁺. It has been demonstrated that Li⁺ does not change Ca²⁺ efflux in rat uterus (6, 7). These results suggest that Li⁺ inhibits smooth muscle contraction without changing Ca²⁺ movements across the plasma membrane. In contrast to the results obtained in the guinea pig ileum, it has been reported that cytosolic Li⁺ inhibited 45Ca²⁺ accumulation in the guinea pig taenia coli (9). The reason for this discrepancy has yet to be clarified.

High K⁺ induced transient increases in [Ca²⁺], and contraction followed by sustained increases in [Ca²⁺], and contraction. In the presence of high K⁺, MLC phosphorylation transiently increased and then decreased to a level near the resting level. This result is similar to the observation by Himpens et al. (29). Our results showed that Li⁺ inhibited the high K⁺-induced increase in MLC phosphorylation. These findings suggest that Li⁺ inhibits the Ca²⁺/calmodulin/MLC kinase system or activates MLC phosphatase. Li⁺ completely inhibited MLC-phosphorylation stimulated by high K⁺, although the high K⁺-induced contraction still remained. This result suggests the existence of a contractile mechanism that does not involve MLC-phosphorylation and that is not inhibited by Li⁺. A further study is necessary to clarify this point.

In the next step, we examined the effect of Li⁺ on calmodulin activity. It is well known that the (Ca²⁺ + Mg²⁺)-ATPase activity of erythrocyte membranes is regulated by Ca²⁺ and calmodulin. In the present study, the calmodulin inhibitors, trifluoperazine and W-7, inhibited
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(Ca²⁺ + Mg²⁺)-ATPase activity in a dose-dependent manner. However, Li⁺ had no effect on the activity. As there is some calmodulin contamination in the erythrocyte membrane preparation, we examined the effect of Li⁺ on the activity without the addition of calmodulin. Although trifluoperazine and W-7 completely inhibited the activity, Li⁺ had no effect on the activity (data not shown). These results suggest that Li⁺ does not act by inhibiting calmodulin activity. In addition, Li⁺ did not change the phosphatase activity as assessed by measuring the rate of relaxation in permeabilized muscle, which reflects the rate of dephosphorylation of MLC (30), or by measuring the dephosphorylation of phosphorylated MLC induced by the crude phosphatase preparation. These results suggest that Li⁺ may directly inhibit the MLC kinase activity or actin-myosin interaction.

It has been reported that MLC kinase thiophosphorylates MLC in the presence of Ca²⁺ and ATPγS (31). Since MLC phosphatase does not reverse the thiophosphorylation, subsequent addition of ATP induces contraction even in the absence of Ca²⁺. This allows us to separate MLCK activity from the actin-myosin interaction (21, 31). Li⁺ (30 mM) did not inhibit the ATP-induced contraction in the thiophosphorylated muscle, suggesting that Li⁺ does not inhibit the actin-myosin interaction. On the other hand, 30 mM Li⁺, added prior to the thiophosphorylation, significantly inhibited the contraction induced by subsequent addition of ATP. Furthermore, Li⁺ decreased the rate of MLC phosphorylation induced by crude MLC kinase. These results suggest that inhibition of MLC phosphorylation is attributable to inhibition of MLC kinase activity.

Li⁺ belongs to group IA ions in the periodic table having similar characteristics to Na⁺. However, Li⁺ has been shown to have various pharmacological actions not shared with Na⁺ (see introduction). Several mechanisms have been proposed. One possibility is that Li⁺ may compete with Mg²⁺ (32). For example, inositol monophosphatase, which requires Mg²⁺ for its activation (33), is inhibited by Li⁺. The other possibility is that Li⁺ somehow blocks calmodulin binding of enzymes. Mork and Geisler have reported that Li⁺ might influence the interaction of calmodulin with the enzyme and/or interfere with the divalent cation site on the adenylate cyclase (4). In our study, the inhibitory action of Li⁺ on contraction in permeabilized muscle was reversed by the addition of excess Ca²⁺ or calmodulin. K252a, which competitively inhibits ATP binding to the catalytic subunit of MLC kinase (26, 27), also inhibited the Ca²⁺-induced contraction in permeabilized muscle, but this effect was not reversed by excess Ca²⁺ and calmodulin. These results suggest that Li⁺ may act by binding to the calmodulin-binding domain of MLC kinase rather than by competing with Mg²⁺ in the cation binding site of the ATP binding domain. However, this mechanism can not apply for all enzymes, since Li⁺ did not inhibit calmodulin activation of the erythrocyte (Ca²⁺ + Mg²⁺)-ATPase. Further study is necessary to clarify the mechanism of Li⁺-induced pharmacological actions.

In conclusion, Li⁺ did not change the cytosolic Ca²⁺ level, calmodulin activity, phosphatase activity and actin-myosin interaction. Li⁺ may inhibit the smooth muscle contraction by an inhibition of MLC kinase in guinea pig ileal smooth muscle.

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