Species Differences in Inhibitory Effect of Li\(^+\) on High K\(^+\)-Induced Contraction in the Ileal Longitudinal Smooth Muscle

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Abstract—The effects of Li\(^+\) substitution for external Na\(^+\) in the medium on a 60 mM KCl (60K\(^+\))-induced contraction were compared in the ileal longitudinal smooth muscles isolated from monkey, rabbit, guinea pig and rat. Li\(^+\) substitution inhibited the 60K\(^+\)-induced contraction in the ileum of all the animal species. Greater inhibitory effect was observed with the increase in external Li\(^+\) concentration and the increase in incubation period with Li\(^+\). Intracellular Li\(^+\) contents of these muscles also increased with the increase in external Li\(^+\) concentration and the incubation period. Thus, a good correlation was obtained between Li\(^+\)-induced inhibition of 60K\(^+\)-induced contraction and the increase in Li\(^+\) content. However, close examination revealed that the contraction in monkey and rat ileum was inhibited by much lower Li\(^+\) accumulation than that in guinea pig and rabbit ileum, suggesting the existence of species difference in the sensitivity of the ileal smooth muscle to the inhibitory effect of Li\(^+\) substitution, and a mechanism for the species differences in the inhibitory action of Li\(^+\)-substitution was discussed.

Lithium ion (Li\(^+\)) belongs to group IA ions in the periodic table having similar physico-chemical characteristics to Na\(^+\). It has been reported that Li\(^+\) enters cultured mouse neuroblastoma cells through Na\(^+\) channels (1) and replacement of external Na\(^+\) with Li\(^+\) results in a loss of intracellular Na\(^+\) and an accumulation of Li\(^+\) since Li\(^+\) is not extruded by the Na\(^+\) pump (2). In guinea pig taenia coli, Li\(^+\) substitution for external Na\(^+\) in the medium caused a membrane depolarization (3) and a contraction (4). In the taenia depolarized with 60 mM KCl (60K\(^+\)), Li\(^+\)-substitution inhibited the muscle contraction (5, 6) without changing the membrane potential (5). Li\(^+\) substitution also inhibited the 60K\(^+\)-induced tonic contraction in the ileal longitudinal muscle, portal vein and uterus in guinea pig (7).

Ouabain, a specific inhibitor of Na\(^+\),K\(^+\)-ATPase, increases intracellular Na\(^+\) and inhibits 60K\(^+\)-induced sustained contraction in guinea pig taenia coli (8, 9) and ileal longitudinal smooth muscle from various animal species (9, 10). In the ileal muscles, the ouabain-induced relaxation showed a species difference in two aspects: the inhibition by ouabain of Na\(^+\),K\(^+\)-ATPase of the muscle and the sensitivity of muscle to accumulated Na\(^+\) (10).

In order to know if there is a similar species difference in the sensitivity of smooth muscle to the inhibitory effect of intracellular Li\(^+\), we compared the Li\(^+\)-induced relaxation and intracellular Li\(^+\) concentration in the ileal longitudinal muscle isolated from monkey, rabbit, guinea pig and rat.

Materials and Methods

Preparations: The experimental animals used were crab-eating monkey (Macaca fasicularis) (about 2 kg), rabbit (about 3 kg), guinea pig (300–400 g) and rat (250–350 g). Monkey was anaesthetized with sodium pentobarbitral (25 mg/kg) and bled, and other animals were sacrificed by a blow on the neck. After exsanguination, the abdomen was opened, and the ileum was removed. The
lower part of the ileum, with 10 cm in distance from the opening of the caecum, was discarded. The ileal longitudinal smooth muscle preparations were made as described by Paton and Aboo Zar (11).

**Solutions:** Physiological salt solution (PSS) had following composition (mM): NaCl, 136.9; KCl, 5.4; CaCl₂, 2.5; MgCl₂, 1.0; NaHCO₃, 11.9 and glucose, 5.5. Li⁺-substituted solution (27.4–91.2 mM) was made by substituting LiCl for an equimolar NaCl in PSS. A Na⁺ solution to wash out extracellular Li⁺ contained (mM) NaCl, 169.5; CaCl₂, 2.5; MgCl₂, 1.0; NaHCO₃, 11.9 and glucose, 5.5.

**Muscle tension measurement:** The contractile tension of muscle strips was isometrically recorded. One end of the strip was tied to a glass holder with nylon thread, and the other end was connected with thread to a strain-gauge transducer (Nihon Kohden). The muscle was suspended in an organ bath containing 15 ml of PSS at 37°C and saturated with 95% O₂ 5% CO₂ at pH 7.2. After equilibration for 30 min, 1 g tension was applied to the monkey, rabbit, guinea pig and rat ileum and about 0.5 g tension in the rat ileum; and 30 min later, 60K⁺ was applied to the muscles. The tonic muscle tension in response to 60K⁺ was considered as a reference response (100%).

**Determination of Li⁺ content:** Intracellular Li⁺ content was determined as reported by Kishimoto and Urakawa (5). After incubation with various test solutions, the strips were exposed to the Na⁺ solution at 0.5°C for 30 min to wash out extracellular Li⁺. After the wash-out, the strips were blotted between filter paper and ashed in a quartz tube with 0.5 ml of a mixture of equal amounts of HNO₃ (61%) and HClO₄ (60%) overnight at 180°C (12). The dried samples were then dissolved in 4 ml of 0.01N HCl. CsCl (1000 ppm) was added to the standard or diluted solution to inhibit the mutual interference of Na⁺. Li⁺ content in the samples was measured by flame photometry (Hitachi), and the results were expressed as in mmole/kg wet wt. of the tissue.

**Results**

**Effects of Li⁺ substitution on 60K⁺-induced contractions:** The ileal longitudinal smooth muscle of monkey, rabbit, guinea pig and rat showed spontaneous mechanical activity. As shown in Fig. 1, 60K⁺ induced a rapid phasic contraction followed by a tonic one in these muscles. The 60K⁺-induced tonic contraction in monkey, rabbit, guinea pig and rat was about 2.5, 2, 4 and 1 g, respectively. After a 30 min-incubation with 60K⁺ solution, external Na⁺ was replaced with Li⁺. A half replacement of Na⁺ with Li⁺ (68.4 mM) gradually decreased the 60K⁺-induced tonic contraction, and the muscle tension remaining after a 60 min incubation was 11.1±1.3, 30.8±2.9, 29.3±2.5 and 21.4±2.4% of each control in monkey, rabbit, guinea pig and rat ileum, respectively. As shown in Fig. 2, the inhibitory effects were dependent on both the concentration of external Li⁺ and the exposure time. Extracellular Li⁺ concentration to induce 50% inhibition (IC50) was 43.9 mM in the guinea pig muscle. IC50 values for extracellular Li⁺ concentration in the other animal species were not calculated because the effect of Li⁺ did not reach equilibrium within a 60 min observation period.

**Changes in intracellular Li⁺ content:** The changes in intracellular Li⁺ content induced by Li⁺ substitution for Na⁺ in 60K⁺ solution are shown in Fig. 3. In the muscle of all animal species, the intracellular Li⁺ content was increased with the increase in the external Li⁺ concentration and also with the exposure time. In the monkey, rabbit, guinea pig and rat ileum, the Li⁺ contents after a 60 min incubation with Li⁺ substituted solution (68.4 mM) containing 60 mM KCl were 6.9±0.6, 10.9±
0.5, 10.6±1.0 and 6.6±0.3 mmole/kg wet wt., respectively. When the 60K+-induced contraction was inhibited to 50% by Li+ substitution, intracellular Li+ content was approximately 8 mmole/kg wet wt. in guinea pig muscle.

**Relationship between the inhibition of tension and intracellular Li+ content:** Correlation between Li+-induced inhibition of muscle tension (Fig. 2) and intracellular Li+ content was shown in Fig. 3. It is clearly shown that the Li+-induced decrease in muscle tension correlates with the increase in Li+ content. The correlation index (r) of the ileal muscle in monkey, rabbit, guinea pig and rat were 0.95, 0.87, 0.91 and 0.87, respectively. The value of the regression coefficient in the muscle of monkey, rabbit, guinea pig or rat was 2.2, 1.0, 1.0 or 1.9, respectively.

**Discussion**

In the present data, Li+ substitution for external Na+ inhibited the 60K+-induced sustained contraction and increased the intracellular Li+ content in the ileal longitudinal muscle isolated from monkey, rabbit, guinea pig and rat. A similar result has been shown in the guinea pig taenia coli (7). Both the Li+-induced inhibition and Li+ content increased with the increase in external Li+ and also with the increase in the Li+-

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![Graphs showing concentration-dependent inhibition of 60K+-induced contraction by Li+ substitution for Na+ in the ileum of monkey, rabbit, guinea pig or rat.](image)
incubation period. After a 60 min incubation with 68.4 mM Li⁺ (a half replacement for Na⁺) solution, monkey and rat ileum accumulated less Li⁺ than the guinea pig and rabbit ileum, whereas the contractions in monkey and rat ileum were more strongly inhibited than those in guinea pig and rabbit ileum. In guinea pig taenia coli, a correlation has been found between Li⁺-induced inhibition and Li⁺ content (5, 7) as well as in the case of Na⁺ (8, 9, 13). In the present study, a good correlation was found between the Li⁺ substitution-induced inhibition of 60K⁺-induced contraction and the intracellular Li⁺ content in the ileal muscles of all the animal species examined. The value of the regression coefficient indicates that the sensitivity of monkey and rat ileum to the inhibitory effect of Li⁺ is much higher than that of guinea pig and rabbit ileum.

A previous report (10) has shown that ouabain induced rapid and larger increase in intracellular Na⁺ content in the ileal muscle of rabbit, dog or guinea pig than that of monkey or rat, that the intracellular Na⁺ inhibits the 60K⁺ induced contraction in the ileum, and that the sensitivity of monkey and rat ileum to the inhibitory effect of intracellular Na⁺ was higher than that of rabbit, guinea pig or dog ileum. Comparing these and the present results, it was found that the ileum
which is sensitive to Na⁺ inhibition is also sensitive to Li⁺ inhibition, as shown in Fig. 5. These results suggest that intracellular Li⁺ has a similar mechanism of inhibition as that of intracellular Na⁺.

It has been shown that the tonic component of 60K⁺-induced contraction is due to the increase in Ca²⁺ influx across the membrane in guinea pig taenia coli (14–16). It has also been shown that Li⁺-induced relaxation is antagonized by the increase in external Ca²⁺ and that intracellularly accumulated Li⁺ inhibits ⁴⁵Ca uptake, suggesting that both cellular Li⁺ and Na⁺ inhibits the high K⁺-induced increase in Ca²⁺ influx in smooth muscle (5, 7, 8). However, when the intracellular free Ca²⁺ level and contractile tension were simultaneously recorded in guinea pig ileal strips loaded with a Ca²⁺ indicator, fura 2, Li⁺ substitution inhibited the 60K⁺-induced contraction but not the intracellular free Ca²⁺ level (17). Moreover, in chemically skinned fiber of guinea pig ileum, the Ca²⁺ (10⁻⁶ M)-induced contraction was inhibited by Li⁺ and
Na\textsuperscript{+} in concentrations needed to induce a 50% inhibition of tension in intact tissue of guinea pig ileum (M. Hori et al., unpublished observation). These results suggest that the inhibition of the contractile protein system was more dominant than that of Ca\textsuperscript{2+} influx in the Li\textsuperscript{+} substitution-induced inhibition of 60K\textsuperscript{-}-induced contraction.

There are some differences between the inhibitory effect of Li\textsuperscript{+} substitution and Na\textsuperscript{+} on 60K\textsuperscript{-}-induced contraction in intestinal smooth muscle: 1) In cat ileal muscle, 60K\textsuperscript{-}-induced contraction was inhibited by intracellular Li\textsuperscript{+}, but not by intracellular Na\textsuperscript{+} (18). 2) In guinea pig ileum, increase in external Ca\textsuperscript{2+} noncompetitively antagonized the effect of Li\textsuperscript{+} (M. Hori et al., unpublished observation) and competitively antagonized the effect of Na\textsuperscript{+} (10). These differences may indicate that the inhibitory mechanism of Li\textsuperscript{+} is partly different from that of Na\textsuperscript{+}. Alternatively, since the increase in Na\textsuperscript{+} content was induced by ouabain, this cardio-tonic agent may have an additional effect on the increase in Na\textsuperscript{+} content, and this additional effect may modify the inhibitory effect of intracellular Na\textsuperscript{+}.

In summary, the 60K\textsuperscript{-}-induced contraction of ileal longitudinal smooth muscles isolated from monkey, rabbit, guinea pig and rat were inhibited by Li\textsuperscript{+} substitution. There is a species difference in the sensitivity of the ileum to the inhibitory action of Li\textsuperscript{+} substitution, which coincides with the species difference in the inhibitory action of intracellular Na\textsuperscript{+}.

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