EFFECTS OF Li-SUBSTITUTION ON HIGH-K INDUCED CONTRACTIONS OF VARIOUS SMOOTH MUSCLE TISSUES IN THE GUINEA-PIG

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Abstract—The effects of partial Li-substitution for external Na in the medium on contraction, cellular Li concentration, and ^{45}Ca uptake of various smooth muscle tissues depolarized by a high-K (62.7 mM) solution were studied. In the taenia coli and ileum isolated from the guinea-pig, Li-substitution (23.7 mM–59.4 mM) increased the cellular Li concentration and strongly inhibited the K-contraction. A good correlation between cellular Li concentration and inhibition of tension was obtained in both the taenia coli and ileum. In the portal vein and uterus, Li-substitution inhibited K-contraction in correlation with Li-accumulation, although the inhibitory effect was weaker than those obtained with the taenia coli and ileum. In the aorta, Li-substitution did not affect K-contraction in spite of the accumulation of Li. The inhibitory effects of Li-substitution in all the tissues except the aorta were antagonized by an increase in external Ca, and the inhibition was accompanied by a decrease in cellular ^{45}Ca content. Although there is a tissue-specific difference, a possibility exists that excess cellular Li inhibits the K-induced Ca influx. The K-induced Ca influx in the aorta seems to be almost insensitive to the inhibitory effect of cellular Li.

Previous studies have shown that partial Li substitution for external Na in the medium can inhibit the tonic contraction of guinea-pig taenia coli induced by high K (1–4). It has also been reported that the Ca-induced contractile response was inhibited by a partial substitution of Li for Na in high-K solution in the longitudinal and circular muscles of guinea-pig stomach (5) and in the rat uterus (6, 7). In our recent paper (8), we reported that Li-induced relaxation of the guinea-pig taenia coli in high-K solution was closely correlated with an increase in cellular Li. The observation suggests that the

increased intracellular Li in some way inhibits the high-K induced inward movement of Ca. Therefore, there may be also a correlation between the increase in cellular Li and the inhibition of K-contraction in different smooth muscle tissues.

In the present study, we clarify the properties of the mechanical response in the guinea-pig taenia coli, ileum, portal vein, aorta, and uterus to various K-concentrations. We have investigated the effect of partial Li-substitution on the 62.7 mM K-induced contraction in these muscles, and the results are compared with each other. Experiments have been performed to study tension development, extracellular space, dry weight/wet weight, cellular Li concentration, and

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^{45}Ca uptake following the treatment with Li. Some important and interesting differences are detected in the various types of smooth muscle preparations.

MATERIALS AND METHODS

Most of the materials and methods were the same as described in our preceding paper (9). The muscle preparations used were the taenia coli, ileum, portal vein, aorta, and uterus isolated from the guinea-pig. Normal physiological salt solution (PSS) contained (mM): NaCl, 136.8; KCl, 5.4; CaCl_2 , 2.5; MgCl_2 , 1.0; NaHCO_3 , 11.9, and glucose, 5.5. In the experiment with varying concentrations of external K, the external Na concentration was modified by substituting Na salts by an equivalent amount of K salts. In most experiments, however, the high-K (62.7 mM) solution was made by increasing KCl to 62.7 mM and reducing NaCl to 106.8 mM. An application of Li was made by substituting LiCl for an equivalent amount of NaCl in the high-K solution.

The contractile tension of muscle strips was isometrically recorded with a strain-gauge transducer (Nihon Kohden). After incubation with the test solution, the muscles

were exposed to a Na-solution (NaCl , 169.5; CaCl_2 , 2.5; MgCl_2 , 1.0; NaHCO_3 , 11.9 and glucose, 5.5 mM) at 0.5°C for 30 min in order to remove extracellular Li. The size of the extracellular space (ECS) was determined by the uptake of an extracellular space maker, ^{14}C -sorbitol (2 $\mu\text{Ci}/\text{ml}$, The Radiochemical Centre, England). Tissue Li content was measured by flame photometry using a method similar to that described by Kishimoto and Urakawa (8), and cellular Li concentration was estimated from the values of ECS and dry weight/wet weight. ^{45}Ca uptake into the muscle cells was measured by a modified "La-method" which was developed by Karaki and Weiss (10). The drugs used were verapamil (Eisai), atropine sulphate, propranolol hydrochloride (Tokyo Kasei), phentolamine methyleate (Ciba Geigy), and β -estradiol (Sigma).

RESULTS

A. Responses to varying concentrations of external K: The muscle strips of the taenia coli, ileum, portal vein, and uterus have spontaneous mechanical activity, but that of the aorta does not (Fig. 1). The spontaneous activity of each muscle in normal solution

Fig. 1. Effects of Li-substitution (59.4 mM) on K-contractions in the taenia coli, ileum, portal vein, aorta, and uterus.

consists of a phasic contraction occurring at almost regular intervals, and the magnitude of the phasic contraction increased by slightly raising external K. Further raising external K resulted in a tonic contraction subsequent to the phasic contraction. On the other hand, the aorta generated a tonic contraction without a phasic one. As shown in Fig. 1, exposure of the taenia coli, ileum, portal vein, and uterus to high-K (62.7 mM) solution resulted in a rapid phasic contraction

(within 1 min) followed by a tonic contraction. The magnitude of the tonic contractions was about 8, 4, 0.7, and 1 g, respectively. In the case with the aorta, the excess K produced a gradual contraction reaching a maximum (about 1 g) after 20 min (Fig. 1). When high-K solution was exchanged with normal PSS or with a Ca-free high-K solution, the developed tension declined to the original level.

The effects of solutions containing 5.4 to

Fig. 2. Effects of excess K on the mechanical activities of the taenia coli, ileum, portal vein, aorta, and uterus. The solution of excess K was prepared by replacing NaCl or NaHCO_3 with equivalent amounts of KCl or KHCO_3 up to 154.1 mM, isotonicity. Square symbols represent the mean values of spontaneous contractions for 20 min. Circle symbols represent the magnitude of tonic contraction. In the ileum and uterus, 20.3 mM K enhanced the spontaneous contraction, but did not produce a tonic contraction. Ordinate: tension (g). Abscissa: external K concentration (mM). Given are the mean values $\pm$ S.E.M. as vertical bars of 4–6 experiments.

154.1 mM K on the mechanical activity in various smooth muscle preparations of the guinea-pig were investigated. Figure 2 shows the magnitude of the tonic contraction obtained by raising external K isotonicity in the taenia coli, ileum, portal vein, aorta, and uterus. Also, tension curves relative to the hypertonic K concentration (10–80 mM) were almost superimposed on those of the isotonic examination, although the results are not shown. In the taenia coli and ileum, maximal tonic contractions (about 10 g and 4.5 g, respectively) were produced by external K concentration in the range of 20 to 35 mM. Progressive elevation in K concentration (60–120 mM) resulted in a concomitant depression of the tonic component. These observations may be explained tentatively by partitioning the source of Ca necessary for the K-induced tonic contraction to two processes: 1. activation of the Ca channel involved in spike generation and 2. the influx of extracellular or superficially bound Ca following depolarization of the membrane (i.e., depolarization induced Ca influx). At a higher K concentration than 60 mM, the Ca spike disappears beyond the threshold of the depolarization block (11). Therefore, such a contraction is smaller than that associated with the high spike frequency in 20–35 mM K.

In the portal vein and aorta the K-induced contractions were graded in the range of 20–60 mM, reaching the maximum (about 0.7 g and 1.2 g, respectively) between 60–120 mM. It is likely that these vascular smooth muscle tissues have little or no contribution from the Ca spike during the K-contraction. In the uterus, the maximal tension (about 4 g) was, similarly to intestinal smooth muscles, produced in the range of 20 to 35 mM K, but further progressive elevation of K concentration completely suppressed the tonic contraction. The maintained tension response of the uterus

may be dependent on Ca spike discharges rather than the depolarization induced Ca influx.

Furthermore, in all the smooth muscles, a complete substitution of K for Na attenuated the developed tonic contraction, and almost no antagonization of the inhibition was obtained by raising the external Ca up to 10 mM. These inhibitions may be attributable to a Na deficiency, according to the suggestion that the Na deficiency causes an inhibition of glucose utilization in the taenia coli (12) or swelling in the aorta (13), leading to muscle relaxation. From the above, it appears that the smooth muscle tissues of functionally different types show a wide range variability with respect to the sensitivity of muscle tension to excess K. Therefore, we have made an investigation using the K-contraction induced by 62.7 mM K (118.7 mM NaCl) in order to minimize the contribution of the Ca spike and to evaluate K-depolarization induced Ca influx.

Furthermore, the effects of various concentrations of K were investigated in the presence of cholinergic and adrenergic antagonists since high K is known to release neurotransmitters from nerve endings. The tension changes by high K were almost unaffected by atropine (2×10^{-6} M), phentolamine (2×10^{-6} M), and propranolol (2×10^{-6} M) in all the preparations used.

B. Effect of Li-substitution on K-contractions: The effect of partial Li-substitution for external Na on the tonic contractions of various smooth muscle tissues depolarized by high-K (62.7 mM) solution was investigated. Half the concentration of external Na was replaced isosmotically by Li (59.4 mM) after a 30 min-incubation with high-K solution. These results are shown in Fig. 1. The Li-substitution gradually decreased the developed contractions of the taenia coli, ileum, portal vein, and uterus to 3.9 ± 0.7 , 15.0 ± 1.3 , 20.0 ± 2.3 , and $30.0 \pm$

2.9% ($n=6$) of each control at 50 min, respectively. As shown in Fig. 3, the inhibitory effects were concentration-dependent (23.7 mM – 59.4 mM). In the case with the aorta, however, the Li-substitution failed to inhibit the K-induced contraction or even contractions developed by K concentrations below 62.7 mM.

C. The effects of Li-substitution and verapamil on Ca-induced contractions: The inhibitory effects of Li-substitution on Ca-induced contractions in high-K solution were investigated. In Fig. 4, these results

are compared with those of verapamil, an organic Ca antagonist. Cumulative addition of Ca (0.25 mM – 10.0 mM) produced graded contractions in all the muscles in Ca depleted high-K medium, suggesting that the K-contractions are strictly external Ca-dependent. Li-substitution was applied 90 min before and during the application of Ca, while verapamil was applied 20 min before. In the taenia coli, ileum, portal vein and uterus, Li-substitution (35.6 mM) shifted the Ca concentration-response curves towards the right. Also, verapamil (5×10^{-8}

Fig. 3. Concentration-dependent inhibition of K-contraction by Li-substitution in the taenia coli, ileum, portal vein, and uterus. Ordinate: relative tension (%) of control. Abscissa: time (min) of exposure to Li (▲), 23.7 mM; (●), 35.6 mM; (■), 59.4 mM. Given are the mean values $\pm$ S.E.M. for 4–6 experiments.

Fig. 4. Effects of Li-substitution and verapamil on the contraction evoked by adding Ca-cumulatively to the various tissues incubated in Ca-free high-K solution for 2 hr. The relative tension expressed as a percent of the K-contraction (Ca, 2.5 mM), is plotted on the ordinate as a function of external Ca concentration on the abscissa. The tissues were pretreated with Li-substitution ($\blacktriangle$, 35.6 mM) for 90 min or with verapamil ($\bullet$, 5×10^{-6} M) for 20 min. The control points are represented by open circles. Given are the mean values $\pm$ S.E.M. as vertical bars of 4–12 experiments.

M) showed similar results in all the muscles. Therefore, the inhibitory effect of Li-substitution is antagonized by external Ca, probably indicating that it interacts at a site directly involved with Ca binding and (or) translocation. This site is presumably the Ca channel which is activated by high-K induced depolarization.

D. Relationship between cellular Li and tension: Possible changes in the size of the extracellular space (ECS) were investigated. As shown in Table 1, the size of the ECS in all the muscle preparations was almost

unaffected by 59.4 mM Li-substitution in high-K solution. In high-K solution, the dry weight/wet weight ratios in the taenia coli, ileum, portal vein, aorta, and uterus were 21.9 ± 0.2 , 21.4 ± 0.2 , 25.7 ± 0.7 , 25.0 ± 1.3 , and $20.0 \pm 1.3\%$ ($n=5$), respectively.

The changes in cellular Li concentration of these muscles following 50% replacement of Na with Li in high-K solution were investigated. As shown in Fig. 5A, the Li-substitution produced a gradual increase in cellular Li in all the preparations examined. After 250 min, the maximum concentration

Table 1. Effects of Li-substitution on the size of the ECS during K-constrictions in the taenia coli, ileum, portal vein, aorta, and uterus. The tissues were incubated in high-K solution with or without 59.4 mM Li for 60 min. For the last 20 min, they were exposed to the radioactive solution with ^{14}C -sorbitol. Numbers in the parentheses indicate the number of experiments. Given are the means $\pm$ S.E.M. There were no significant differences between the sizes of the ECS of the tissues incubated in high-K solutions with and without Li.

Extracellular space (^{14}C -sorbitol, %)			
	Normal PSS	High-K solution	High-K+Li
	(4)	(8)	(4)
Taenia coli	31.2 $\pm$ 1.5	33.7 $\pm$ 1.9	35.2 $\pm$ 0.9
Ileum	34.5 $\pm$ 1.5	35.3 $\pm$ 1.9	41.3 $\pm$ 1.9
Portal vein	48.4 $\pm$ 2.9	55.5 $\pm$ 1.8	60.7 $\pm$ 3.4
Aorta	57.7 $\pm$ 1.5	56.2 $\pm$ 1.4	54.8 $\pm$ 3.0
Uterus	54.2 $\pm$ 2.2	51.7 $\pm$ 2.7	57.3 $\pm$ 1.4

of Li accumulation reached in the taenia coli, ileum, portal vein, aorta, and uterus were 71.8 ± 4.5 , 65.8 ± 4.6 , 73.8 ± 5.2 , 69.1 ± 2.1 , and 58.0 ± 4.6 mmol/l cell water ($n=4$), respectively. Figure 5B demonstrates the relationship between the increase in cellular Li and tension. In the taenia coli, ileum, portal vein, and uterus, a correlation was obtained between cellular Li concentration and inhibition of tension. The cellular Li concentrations to inhibit K-constrictions of the taenia coli, ileum, portal vein, and uterus by 50% were approximately 21.2, 19.0, 32.5, and 56.5 mmol/l cell water, respectively. In the aorta, however, no correlation was seen.

E. Change in cellular ^{45}Ca content: The effect of Li-substitution on the K-induced increase in cellular Ca content was investigated. These results are shown in Fig. 6. Muscle strips were incubated in high-K solution with or without 59.4 mM Li for 60 min, and then they were transferred to the radioactive solution. After loading with ^{45}Ca for 30 min, the cellular ^{45}Ca content was measured by a modified "La-method". In high-K solution, ^{45}Ca uptake of the taenia coli, ileum and aorta increased significantly compared with those in normal solution. In the case with the uterus, we

Fig. 5. A: Changes in cellular Li concentration induced by Li-substitution in K-depolarized preparations of the taenia coli (●), ileum (○), portal vein (▲), aorta (△), and uterus (■). Ordinate: cellular Li concentration (mmol/l cell water). Abscissa: time (min) of exposure to 59.4 mM Li. B: Relationship between cellular Li concentration and tension. Ordinate: relative tension (%). Abscissa: cellular Li concentration (mmol/l cell water). The symbols are the same as for A. Given are the mean values $\pm$ S.E.M. for 4–8 determinations.

Fig. 6. Effect of Li-substitution on ^{45}Ca uptake during K-contraction in the taenia coli, ileum, aorta, and uterus. The tissues were treated with 59.4 mM Li for 60 min before and during exposure to the radioactive solutions. The ^{45}Ca uptake period was 30 min. The vertical bars represent $\pm$ S.E.M. for 4–8 determinations.

*: Significantly different from the value in high K solution ($P < 0.05$).

could not obtain a significant increase in ^{45}Ca content by high-K solution because of the large variation. The K-induced ^{45}Ca uptake was attenuated by Li-substitution in the taenia coli, ileum and uterus but not in the aorta. These results seem to be in good accord with those of the tension experiment. The result with the portal vein could not be shown because of the scattering of the data obtained.

DISCUSSION

Previous studies have shown that when Li is partially substituted for Na in high-K solution, the developed tension of guinea-pig taenia coli gradually decreased to the original level (1–4). It has also been reported that the Ca-induced contractile response was inhibited by a partial substitution with Li for Na in high-K solution in the longitudinal and circular muscles of guinea-pig stomach (5) and in rat uterus (7). In addition, Freer and Smith (6) reported that Li inhibited angiotensin II and acetylcholine contraction,

suggesting that Li may be a specific inhibitor in uterine tissue.

Our recent study (8) suggests that Li-substitution which inhibits the tonic contraction of the taenia coli depolarized by high K has the following properties: 1) no effect on K-induced membrane depolarization 2) the dependence of the inhibitory effect on the concentration of Li-substitution, 3) the dependence on the length of the period of application of Li-substitution, 4) the correlation between the accumulation of cellular Li and the inhibition, 5) the antagonization by raising external Ca and 6) the inhibition of ^{45}Ca uptake. These findings indicate that the action of Li is closely related to an accumulation of intracellular Li; the possible mechanism involved is to inhibit Ca influx in response to depolarization with high K. As shown in the present results, the inhibitory effect of Li-substitution was antagonized by raising external Ca concentration in guinea-pig taenia coli, ileum, portal vein, and uterus. It was also associated with a decrease in cellular ^{45}Ca content, although we can not express these relationships quantitatively. Freer and Smith (6, 7) have speculated that extracellular Li, similarly to a Ca antagonist, competes with Ca for binding to negative sites or the cell surface, since an increase in external Ca attenuates the inhibition. However, it is difficult to consider that external Li reduces Ca influx by competing with Ca for influx sites in the membrane since the relaxation caused by Li-substitution was dependent on the duration of the exposure to Li. From the above, a possibility exists that excess cellular Li inhibits K-induced Ca influx in the several smooth muscle tissues of guinea pigs. However, the inability of Li to inhibit K-contraction in the aorta may reflect a basic difference in the mechanisms of Ca utilization in the aorta relative to the other muscles as well as a possible inhibitory action of Li unrelated to

Ca transport ability induced by K-depolarization.

The relationship between cellular Li concentration and relaxation obtained from the experimental results with the ileum resembles closely what was observed in the taenia coli. However, the cellular Li concentration in the portal vein or uterus to inhibit K-contraction by 50% was higher than that required with the taenia coli. It also seems that K-induced Ca influx in the aorta is almost insensitive to the inhibitory effect of cellular Li. Thus, there is a tissue-specific difference in the sensitivity to Li and the order is guinea-pig taenia coli, ileum>portal vein>uterus>aorta. On the other hand, the present results indicate that contractile responses to various K concentrations are rather different among intestinal, vascular, and uterine smooth muscles. These contractile activities seem to be more dependent upon depolarization-induced Ca influx than Ca spike discharge in the following order: uterus>taenia coli, ileum>portal vein, aorta. However, this difference is incompatible with the order of the tissue-specific difference in the sensitivity to Li. We have also reported that the order of the tissue-specific difference in the sensitivity to verapamil is uterus>portal vein, ileum, taenia coli>aorta, reflecting the dependence on external Ca for K (62.7 mM)-contractions of these tissues (9). Therefore, the property of the tissue-specific difference on the inhibitory effect of Li is dissimilar to verapamil, but is similar to the case with ouabain (9).

In the preceding paper (9), we have reported that the ouabain-induced inhibition of K-contractions of the taenia coli, ileum, portal vein, and uterus is explained by the increased intracellular Na which would inhibit the K-induced increase in Ca influx. The cellular Na concentrations to inhibit K-contractions of the taenia coli and ileum by 50% are about 62.5 and 60.0 mmol/l cell

water, respectively. The cellular Li concentrations to inhibit by 50% are about 21.2 and 19.0 mmol/l cell water, respectively. Thus, the inhibitory effect of cellular Li is greater than that of cellular Na. Although the difference of the potency between the two ions is difficult to interpret, the binding tendency of Li by many ligands is preferential to that of Na according to stoichiometric stability constants. Cellular Na and Li appear to interact at a common site (14). If so, the tendency of Li to combine with the sites involved in the inhibitory action would be greater than the tendency of Na.

In conclusion, Li-substitution inhibited K-contraction in guinea-pig taenia coli, ileum, portal vein, and uterus but not in the aorta. The inhibition was closely related to an accumulation of intracellular Li which would cause relaxation by inhibiting K-depolarization induced Ca entry. It is also suggested that the inhibitory effect of cellular Li is greater than that of cellular Na. However, there is a tissue-specific difference in the sensitivity to Li, and the cause should be further investigated.

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