A Comparative Study on the Contraction Induced by High K/Na Deficient Solution in the Trachea, Gall Bladder and Urinary Bladder in Guinea-Pig

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Abstract—In the present paper, a comparative study of high K solution was carried out on the mechanical response, wet and dry weights of tissue, and extracellular space of ^{14}C -sorbitol in the trachea, gall bladder, and urinary bladder of guinea-pig. Hyperosmotic addition of KCl to the physiological solution produced a sustained contraction, while isosmotic substitution of K for Na in the physiological solution induced a contraction followed by a small sustained one in the trachea, gall bladder or urinary bladder. At 120 min after application of the substituted KCl, Na deficient solution, it increased cell water content in the smooth muscle cells of the trachea or gall bladder, but did not change that in the urinary bladder. Both the inhibition of contraction and the swelling in the trachea or gall bladder were prevented by the hyperosmotic application of sucrose or NaCl. The substitution for NaCl with the K salts with more permeable anions ($\text{I}^- > \text{NO}_3^- > \text{Cl}^-$) produced a greater inhibition of contraction in the trachea and gall bladder. Although the impermeable anion $\text{C}_2\text{H}_5\text{CO}_2^-$ did not inhibit the contraction in the trachea, this anion partially inhibited it in the gall bladder. On the other hand, the inhibition of contraction in the urinary bladder was prevented by addition of pyruvate, oxalacetate or NaCl. From these results, it is suggested that the inhibition of contraction in the trachea and gall bladder, quiescent smooth muscle, is probably due to cell swelling and that the inhibition of contraction in the urinary bladder, spontaneously active smooth muscle, is mainly caused by an inhibition of glucose utilization by Na deficiency in the medium. However, the gall bladder probably has an intermediate property between the trachea and urinary bladder in high K-induced contraction.

An elevation of potassium concentration (high K) in a physiological salt solution (PSS) induces a contraction in various smooth muscle preparations. It is well known that the high K solution-induced contraction is caused by membrane depolarization of the muscle and depends on extracellular Ca ion entering through a voltage-sensitive Ca channel. This contraction is usually utilized as a standard contraction for the phar-

macological analysis of a chemical agent on various smooth muscles. The high K solution is prepared either by adding KCl hyperosmotically or by substituting NaCl with isosmolar KCl in the PSS (1).

In guinea-pig taenia coli, hyperosmotical high K solution induced a sustained contraction, while substituted high K solution induced a transient contraction followed by a small sustained one (2, 3). The attenuation

of the sustained contraction in the substituted high K solution is probably attributable to an inhibition of glucose utilization, a decreased energy production, resulting from Na deficiency and partly to a swelling of the smooth muscle of the taenia coli (4, 5). On the other hand, the transient contraction by substituted high K solution is simply due to swelling of the muscle cells in the rabbit aorta (6).

It is generally accepted that there are two groups of smooth muscle, that is, a spontaneously active muscle and a quiescent one. The latter was also classified into two subgroups. A smooth muscle in subgroup-I is evoked to produce a spike by electric stimulation to the muscle or nerve, and a muscle in subgroup-II is not evoked to produce a spike by either stimulus. Guinea-pig taenia coli (7) and rabbit (8) or guinea-pig urinary bladder (9, 10) belong to the group of spontaneously active muscle. The gall bladder of guinea-pig (11) belongs to subgroup-I of quiescent muscle, and rodent aorta (12, 13) and bovine (14, 15) or canine (16–18) trachea belongs to subgroup-II. In the present paper, we compared the effect of high K solution on mechanical response, wet and dry weights of tissue, and extracellular space of ^{14}C -sorbitol in the smooth muscle of the trachea, gall bladder and urinary bladder of guinea-pig.

Materials and Methods

Male guinea-pigs weighing 250 to 300 g were killed by a blow on the head and bled to death. The tracheal muscle was obtained from the extrathoracic cervical trachea. In the ring of trachea with a width of 2–3 mm, cartilage crescents were opened at opposite sides of the smooth muscle layer. Three pieces of trachea were connected to each other with a nylon string at the end of the cartilages. The gall bladder was removed from the opened abdomen by cutting at the distal end cystic duct. The superficial connective tissue was removed, and the preparation was longitudinally quartered as a muscle strip. The removed urinary bladder was opened from the dorsal surface running between the ureter openings to the dome of the bladder or from the mid-ventral posterior region. *Trigomum vesicae*, *muscle sphincter*

vesicae and *Ostium ureteris* were excised from the preparation. The mucous layer and superficial connective tissue were removed (9).

One end of each of the strips was bound to a glass holder with nylon thread, and the other end was connected to a strain-gauge transducer (Nihon Kohden) by thread. The muscle was suspended in an organ bath containing 15 ml of PSS and equilibrated for 60 min. The muscle contraction was isometrically recorded. Strips of trachea or gall bladder were loaded with 0.5 g, and the urinary bladder loaded with 1.0 g. After the 60 min incubation in PSS, 60 mM KCl was hyperosmotically applied twice to the muscle. When the muscle maintained tension and wet weight reached a steady level, various concentrations of KCl were applied. The tension developed by 65.4 mM K at 120 min was referred to as the control (100%).

The PSS employed was a modified Tyrode's solution of the following composition (mM): NaCl, 136.8; KCl, 5.4; CaCl_2 , 2.5; MgCl_2 , 1.0; NaHCO_3 , 11.9 and glucose, 5.5. A hyperosmotic high K solution was prepared by adding an appropriate volume of 3 M KCl stock solution. A substituted high K solution was prepared by adding KCl and substituting equimolar NaCl to maintain isosmolarity. In all substituted high K solutions, 11.9 mM NaHCO_3 was substituted with equimolar KH_2CO_3 . In some experiments, Cl^- was isosmolarilly substituted by $\text{C}_2\text{H}_5\text{CO}_2^-$, NO_3^- or I^- in the high K solution. An appropriate amount of sucrose or NaCl was added to the substituted high K solution to make a hyperosmotic solution in some experiments. These solutions were aerated with a 95% O_2 –5% CO_2 gas mixture at 37°C and pH 7.2.

In the experiment of tissue weights and extracellular space (ECS), a rectangular piece of tracheal smooth muscle was employed. Since the smooth muscle in the trachea was arranged in a broad band which bridges the defect in the cartilage rings, the strips were longitudinally excised (length of 10–15 mm) from the tracheal duct. As it was technically difficult to excise completely the cartilage and connective tissue from the trachea, small amounts of them remained in the muscle preparation. For determination of

wet weight of the tissue, the muscle strips were treated with various test solutions after the equilibration in PSS. During the incubation period, the strips were removed occasionally from the organ bath, blotted on a filter paper to exclude adhering solution and weighed with a balance. The relative wet weight was expressed as wet weight (g)/control wet weight (g). To determine the dry weight of the tissues, the sample was incubated in PSS or test solution for 120 min, and then the muscle was removed, blotted, weighed, dried at 95°C for 20 hr in vessels, and weighed again. For a determination of ECS, muscle preparations after a pre-incubation in PSS were incubated in various test solutions for 120 min. ^{14}C -sorbitol (2

$\mu\text{Ci/ml}$, The Radiochemical Center, England) was added at the last 20 min in the incubation. Then the strips were removed from the bath, blotted on a filter paper, weighed and digested with 0.2 ml of a solubilizer (Lumac solve) at 55–60°C, overnight. The solubilized sample was mixed with 4 ml of scintillator (Lumagel, Lumac Systems A.G.). The radioactivity was counted using a liquid scintillation spectrometer (Tri-Carb 3380, Packard Co.) at room temperature. Assuming that the high K solution does not change the dry weight of the tissue and that the specific gravity of the tissues are regarded as 1, the cell water content was calculated from the following equation:

$$\frac{\text{Wet weight (g) (Treated muscle)}}{\text{Wet weight (g) (Control muscle)}} \times \frac{\{1 - (\text{Dry weight} \div \text{ECS g/Kg Wet weight})\} (\text{Treated muscle})}{\{1 - (\text{Dry weight} \div \text{ECS g/Kg Wet weight})\} (\text{Control muscle})}$$

Drugs employed were tetrodotoxin (Sankyo Co.), scopolamine hydrochloride (Sigma), tripeleennamine hydrochloride (Ciba), potassium propionate (Tokyo Kasei Co.), pyruvic acid (Wako Junyaku Co.), oxalacetic acid (Wako Junyaku Co.) and phlorizin (Sigma).

Values were expressed as a mean $\pm$ S.E.M., and a statistically analysis was performed with Student's *t*-test.

Results

Changes in muscle tension and wet weight of tissue

Trachea: When 65.4 mM KCl (hyper-65K) was hyperosmotically applied to the trachea, the muscle gradually increased its tension which reached maximum at 30 min and was maintained at a steady level during 120 min. Figure 1A shows the statistical result of the time course of tension change. Isosmotic 40, 60 or 120 mM K (iso-40, -60 or -120K) solution also induced a contraction, then it gradually attenuated the muscle tension became 68, 81 or 73% of that by hyper-65K solution at 120 min, respectively. When isosmotic 154 mM K (iso-154K) solution was applied to the muscle, it developed a muscle tension, then it decreased to 13% at 120 min (Fig. 1A). Although hyper-65 or iso-60K solution significantly decreased the relative value for the wet weight of the tissue

at 120 min, iso-154K solution increased it to 1.1 (Fig. 1A'). Concentration-response curves between various concentrations of high K solution and muscle tension or relative wet weight of the tissue are shown in Fig. 2A. It seems that the curve of muscle tension is roughly a mirror image of that for the wet weight of the tissue.

Since these high K-induced contractions in the trachea were not affected by treatment with 10^{-7} g/ml tetrodotoxin (an inhibitor of nerve conductance), 10^{-6} – 10^{-5} M hyoscine (an anticholinergic drug) or 10^{-6} – 10^{-5} M tripeleennamine (a H_1 -receptor antagonist), it seems that the contractions may not be mediated by released nerve transmitters.

Gall bladder: An application of hyper-65K solution to the preparation of gall bladder showed almost similar results in tension and wet weight of tissue to those in the trachea (Fig. 1B, 1B'). Iso-40, -60 or -120 K solution decreased muscle tension to 61, 81 or 59% of that by hyper-65K solution at 120 min, respectively. However, iso-154K solution induced a contraction followed by relaxation which reached to 23% (Fig. 1B). Although iso-40, -60 or -120K solution decreased the relative wet weight, iso-154K solution increased it to 1.1 at 120 min (Fig. 1B'). Concentration-response curves of muscle tension or wet weight of tissue were almost the same type as those of the trachea (Fig.

Fig. 1. Changes in tension (A, B, C) and relative wet weight (A', B', C') of guinea-pig trachea, gall bladder and urinary bladder in high K solutions. Normal Tyrode solution (PSS). Hyperosmotically added 65.4 mM KCl (hyper-65K), isosmotically substituted 60 mM K (iso-60K), 120 mM K (iso-120K) or 154.2 mM K (iso-154K) was applied at 0 min. Muscle tension induced by hyper-65K solution at 120 min was the control (100%). Relative wet weight is expressed as wet weight (g)/control weight (g), and the mean $\pm$ S.E. of six experiments is shown. In some cases, the S.E. was smaller than the size of the symbol.

Fig. 2. Changes in muscle tension (●) and relative wet weight (▲) obtained 120 min after the application of substituted high K solutions. Details are described in Fig. 1. * $P < 0.05$, ** $P < 0.01$: Significant differences from the control (PSS) at 120 min.

2B).

Urinary bladder: The urinary bladder showed a large phasic contraction, followed by tonic one, in each of the high K solutions. The tonic response to iso-40, -60 or -120K solution at 120 min was 58, 70 or 73% of that to hyper-65K solution. However, the tonic response to iso-154K solution started to decrease 30 min after, and it gradually reached to 24% at 120 min (Fig. 1C). These high K-induced contractions were not changed by the treatment with the antagonists mentioned above. The wet weight of the urinary bladder was not affected by iso-154K solution, though its relative wet weight was reduced by hyper-65, iso-40, -60 or -120K

solution at 120 min (Fig. 1C'). Although the concentration-response curves of muscle tension and relative wet weight of the urinary bladder were almost similar in type to those of the gall bladder and trachea, the concentration-response curve of relative wet weight in the urinary bladder showed a smaller change than those in the gall bladder and trachea (Fig. 2C).

Swelling of the muscle preparations in iso-154K solution

Cell water content: The average dry weights of the trachea, gall bladder and urinary bladder in each of six samples in PSS were 181 ± 12 g/kg wet wt., 142 ± 4 g/kg wet wt. and 163 ± 3 g/kg wet wt., respectively.

On the other hand, ECS values of the trachea and gall bladder in the PSS were 558 ± 26 ml/kg wet wt. and 445 ± 15 ml/kg wet wt., respectively, and they decreased to 448 ± 27 ml/kg wet wt. and 330 ± 25 ml/kg wet wt. in iso-154K solution at 120 min, respectively. In the case of the urinary bladder, the ECS in PSS (333 ± 15 ml/kg wet wt.) was not significantly different from that in iso-154K solution (338 ± 20 ml/kg wet wt.) at 120 min. Relative cell water content was calculated from the equation described in "Methods". The relative cell water contents of the trachea or gall bladder in iso-154K solution at 120 min were 1.63 and 1.44, respectively. However the relative value for the urinary bladder was 0.99 in iso-154K solution. From the data, iso-154K solution, which decreased the muscle tension in all the muscles, swelled the trachea and gall bladder, but not the urinary bladder.

Hyperosmotic addition of sucrose: In the trachea, the addition of sucrose at a concentration of 25, 50 or 100 mM to iso-154K solution developed tension to 54, 91 or 89% of that by hyper-65K solution at 120 min, respectively. Concentration-response curves between sucrose concentration and muscle tension or wet weight of the tissue in iso-154K solution are shown in Fig. 3A. Increasing sucrose concentration increased the tension, but decreased the relative wet weight. In the gall bladder, addition of sucrose gave almost the same results as those in the trachea (Fig. 3B). The data showed a reciprocal relationship between muscle tension and wet weight in the trachea or gall bladder. On the other

hand, the decreased tension induced by the iso-154K solution in the urinary bladder was slightly increased by the hyperosmotic addition of sucrose at a concentration of 25 or 50 mM (Fig. 3C). Even the addition of 100 mM sucrose to the urinary bladder prevented the decrease in tension to 48% of that by hyper-65K solution, and the prevention was remarkably smaller than those in the trachea and gall bladder. From the results, it is assumed that the decrease in the tension of the urinary bladder, unlike other tissues, is not mainly due to swelling of the cells.

Anion substitution: In this series of experiments, the effect of an impermeant anion, $\text{C}_2\text{H}_5\text{CO}_2^-$, or permeant ones, Cl^- , NO_3^- or I^- , was compared to iso-154K induced contraction in the trachea or gall bladder. The tracheal muscle maintained the developed tension for 120 min in substituted K propionate solution; however, the tension of the gall bladder decreased to 37% of that by hyper-65K solution at 120 min (Fig. 4A, 4B). By contrast, tension in KNO_3 or KI solution was more rapidly decreased than that in KCl solution in both the muscles. The order of anion permeability ($\text{I}^- > \text{NO}_3^- > \text{Cl}^- > \text{C}_2\text{H}_5\text{CO}_2^-$) corresponded to that of the decrease in high K-induced contraction by anion substitution ($\text{I}^- > \text{NO}_3^- > \text{Cl}^- > \text{C}_2\text{H}_5\text{CO}_2^-$).

The glucose utilization of the muscles during the contraction induced by high K solution: The contraction induced by hyper-65K solution in the urinary bladder was maintained at a maximum level for 120 min. When glucose was removed from the high K

Fig. 3. Changes in tension (●) and relative wet weight (▲) by the addition of sucrose. Muscle strips were treated with iso-154K solution with or without sucrose for 120 min. Details were described in Fig. 1. ★ $P < 0.05$, ★★ $P < 0.01$: Significant difference from the control (154K solution without sucrose at 120 min).

Fig. 4. Changes in muscle tension in iso-154K solution with various anions. Muscle tension induced by hyper-65K solution at 120 min was used as the control (100%).

Table 1. Effects of glucose removal and addition of various substances on high K-induced contraction in trachea, gall bladder and urinary bladder of guinea-pig

	Trachea	Gall bladder	Urinary bladder
Tension (g)			
Hyper 65K	1.49±0.01	2.18±0.01	5.25±0.18
Relative contraction (%)			
Hyper 65K	100	100	100
Glucose removal	98.1±9.1	60.2±5.8	16.1±0.7
Pyruvate (5.5 mM)	—	115.6±8.1	95.3±5.6
Oxalacetate (5.5 mM)	—	—	100.1±5.2
Phlorizin (10 ⁻³ M)	99.0±3.6	64.5±4.4	45.8±4.5
Iso 154K	13.4±1.7	23.3±3.3	24.5±1.5
Sucrose (100 mM)	89.4±6.4	88.5±4.0	48.4±3.6
NaCl (50 mM)	97.8±7.2	69.2±6.3	65.0±5.5
Pyruvate (5.5 mM)	—	36.2±3.8	101.1±6.2
Oxalacetate (5.5 mM)	—	—	90.1±5.6

All the values were obtained 120 min after the application of high K solution with or without glucose. Pyruvate or oxalacetate was simultaneously applied with the glucose-free high K solution. Muscle tension induced by hyper-65K solution at 120 min was used as the control (100%). The mean±S.E. was obtained from 6–8 experiments.

medium, the developed tension gradually decreased, then it reached to 16% at 120 min (Table 1). Addition of sodium pyruvate or oxalacetate at a concentration of 5.5 mM to the high K medium without glucose maintained the muscle tension at the maximal level for 120 min. An application of phlorizin (10⁻³ M), a specific inhibitor of glucose and Na symport (19), decreased the hyper 65K-induced contraction to about half.

The developed tension of the urinary bladder in iso-154K medium decreased to approximately 25% of that in hyper-65K medium. A hyperosmotic addition of 25 or

50 mM NaCl to the iso-154K solution prevented the decrease in the tension to 60 or 65%, respectively. The data showed that the addition of NaCl was more effective than addition of sucrose. On the other hand, the addition of pyruvate (5.5 mM) to iso-154K solution without glucose did not decrease the developed tension in the urinary bladder (Table 1).

In the gall bladder, the glucose removal decreased the contraction in hyper-65K solution to half, and the addition of 5.5 mM pyruvate to the solution without glucose completely prevented the decrease in tension.

The hyperosmotic addition of NaCl (25 or 50 mM) to iso-154K solution was less effective on the prevention than that of sucrose. Moreover, the addition of pyruvate slightly prevented the decreased tension in iso-154K solution without glucose (Table 1). However, in the trachea, the removal of glucose or application of phlorizin (10^{-3} M) did not change the developed tension in hyper-65K solution, which is consistent with the results by Saida et al. (20). Changes in tension of the gall bladder by the glucose removal or by the application of various substances were changes that were almost intermediate between those of the trachea and urinary bladder.

Discussion

Hyperosmotically added K solution induced a sustained contraction, while isosmotically substituted high K, Na deficient solution induced a transient contraction in guinea-pig taenia coli (2–4) and rabbit aorta (6). However, a substituted high K solution induced a sustained contraction during 120 min in bovine (21) and monkey trachea (F. Ueda and N. Urakawa, unpublished data). In the present data, iso-154K (Na deficient) solution induced a maximum contraction, then gradually decreased the muscle tension to 20% at 120 min in the trachea, gall bladder and urinary bladder in guinea-pig.

It was observed that substituted high K solution swelled smooth muscles of the guinea-pig taenia coli (4, 22, 23) and rabbit aorta (6, 22). The swelling of the aorta was almost completely prevented by the hyperosmotic addition of sucrose (6, 22). In the present paper, the trachea or gall bladder was swollen by iso-154K solution. The swelling and the decrease in developed tension in the trachea or gall bladder were completely prevented by the addition of 100 mM sucrose. It seems that the inhibition of contraction in the trachea or gall bladder is caused by cell swelling.

The substitution for NaCl by the K salts with more permeable anions ($I^- > NO_3^- > Cl^-$) produced a greater inhibition of the contraction in rabbit aorta (6). In this data, the effect of substitution with a more permeable anion (I^- or NO_3^-) for Cl^- on iso-154K

induced contraction in the trachea was the same as those in the rabbit aorta (6). On the other hand, the swelling of the aorta was prevented by using a K salt with an impermeable anion, SO_4^- (22) or $C_2H_5CO_2^-$ (6). The present data showed that the K salt with $C_2H_5CO_2^-$ prevented the inhibition of the contraction of the trachea in iso-154K solution. The data suggests that a close relationship between swelling and decrease in muscle tension exists in the trachea treated with substituted solution containing both potassium and chloride ions as well as in the aorta. Both the muscles belong to subgroup-II of quiescent muscle. The gall bladder which belongs to subgroup-I showed similar results in the substitution with more permeable anions to that in the aorta or trachea. However, the substitution with an impermeable anion ($C_2H_5CO_2^-$) did not fully prevent the inhibition of iso-154K induced contraction. Further study is needed to clarify the role of Cl^- in swelling of the gall bladder.

In guinea-pig taenia coli, although the decrease in sustained tension in substituted K, Na deficient solution was reported to be only partially reversed by the hyperosmotic addition of sucrose, such an inhibition of sustained contraction was recovered when 5.5 mM pyruvate or oxalacetate or 50 mM NaCl was added during the contraction (4, 22). Since glucose transport across the cell membrane is probably dependent on external Na in the smooth muscle of taenia coli (4, 5), as seen in the small intestine epithelium and kidney (19), inhibition of contraction may be brought by Na deficiency, resulting in decreased energy production in the taenia coli (4, 5). In the urinary bladder, the removal of glucose from hyper-65K solution attenuated the developed tension. Further, an application of phlorizin at a relative high concentration (10^{-3} M) inhibited hyper-65K induced contraction. The inhibition of contraction induced by iso-154K solution was also prevented by application of pyruvate or oxalacetate and by hyperosmotic application of NaCl, which was more effective than that of sucrose. The data shows that the urinary bladder probably utilizes glucose as an energy source for high K-induced contraction

in the presence of sodium. The urinary bladder as well as the taenia coli is a spontaneously active muscle.

In the gall bladder, although the inhibition of iso-154K induced contraction was caused by swelling and prevented by the application of hyperosmotic sucrose, the hyper-65K induced contraction was attenuated by glucose removal or application of phlorizin. Moreover, the addition of pyruvate slightly prevented the inhibition. Accordingly, it seems that the gall bladder, which belongs to the subgroup-I of quiescent muscle, the high K-induced contractions have properties that are intermediate between those of the trachea, which belongs to subgroup-II of quiescent muscle, and the urinary bladder, a spontaneously active muscle.

In summary, the substituted KCl, Na deficient solution developed a muscle tension, followed by a remarkable decrease at 120 min, in the trachea, gall bladder and urinary bladder of guinea-pig. It is suggested that the inhibition of contraction in the trachea and gall bladder, which are quiescent muscles, is probably due to a cell swelling and that the inhibition in the urinary bladder, a spontaneously active muscle, is mainly caused by an inhibition of glucose utilization caused by Na deficiency in the medium.

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