Study on the Contraction and Oxygen Consumption Induced by High K, Na-Deficient Solution in the Urinary Bladder and Gall Bladder of Guinea-Pig

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Abstract—In urinary bladder and gall bladder of guinea-pig, the application of hyperosmotic 65.4 mM K solution induced a tonic contraction and increased the rate of oxygen consumption two or three times. The increased O₂ consumption maintained steady level during 120 min. In isosmotic 154 mM K, Na-deficient solution, the tonic contraction gradually decreased and oxygen consumption also showed a transient increase followed by a small one. In the urinary bladder, the addition of pyruvic acid completely prevented the decline of the tonic contraction by iso-154K solution at 120 min, but this addition partially prevented it in the gall bladder. Hyperosmotic application of sucrose partially prevented it in the urinary bladder, but completely recovered it in the gall bladder. When NaCl was applied to iso-154K solution, the decrease of tonic contraction was prevented by half in both the muscles. The application of pyruvic acid and NaCl prevented the reduction of oxygen consumption by iso-154K solution in the urinary bladder, but in the gall bladder, this had little effect. There was a close correlation (r=0.950) between the muscle tension and the rate of oxygen consumption in the urinary bladder at 120 min under various conditions. In the gall bladder, a correlation (r=0.875) was also found between both when the results by hyperosmotic addition of NaCl and sucrose were omitted. In the urinary bladder, tension cost, a ratio of O₂ consumption rate (μmol O₂/g/min) to developed tension (kg force/cm²), was large (0.206) and similar to that of taenia coli; and that of gall bladder was small (0.130) and almost similar to that of vascular smooth muscle. In summary, the inhibition of contraction and rate of oxygen consumption induced by high K, Na-deficient solution in the urinary bladder is probably caused by inhibition of Na-glucose symport and similar to that in taenia coli. On the other hand, that of the gall bladder seems to be mainly caused by cell swelling, similar to that of vascular smooth muscle, but partially caused by inhibition of the glucose symport.

It has been reported that hyperosmotically added KCl induced a tonic contraction and isosmotically substituted K, Na-deficient solution induced only a transient contraction in various types of smooth muscles (1-7). Effect of high K, Na-deficient solution was investigated with regards to muscle tension, cell water content, rate of oxygen consumption and ATP content in the smooth muscle of guinea-pig taenia coli (4, 7) and rabbit aorta (5, 7). From these data, it was found that in taenia coli, the high K, Na-
deficient solution depressed ATP synthesis of the muscle probably because Na-glucose symport was prevented by Na deficiency and thus inhibited the tonic contraction (4, 7). However, the high K, Na-deficient solution seemed to inhibit the tonic contraction by swelling of cells in rabbit aorta (5, 7).

On the other hand, it was reported that the tonic contraction induced by high K, Na-deficient solution was also inhibited in guinea-pig urinary bladder or gall bladder (6). From only the data on muscle tension and calculated cell water content, it was assumed that the inhibition of tonic contraction in the urinary bladder might be caused by an inhibition of glucose utilization in Na-deficient medium and that the inhibition in the gall bladder might be mainly caused by cell swelling, but partially caused by the inhibition of glucose utilization.

In the present experiment, we examined contractions and rate of oxygen consumption of guinea-pig urinary bladder or gall bladder in various kinds of high K solution and attempted to confirm the results in the previous paper.

Materials and Methods

Changes in muscle tension: Male guinea-pig weighing 250 to 300 g were stunned by a blow on the head and bled to death. The gall bladder was removed from the opened abdomen and cut at the distal end of the cystic duct. 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. Trigonum vesicae, muscle sphincter vesicae and ostium ureteris were excised from the bladder. Both the preparations after removal of connective tissue were quadrisectioned longitudinally.

The muscle strips were suspended in an organ bath containing 15 ml of physiological salt solution (PSS) with a strain gauge transducer and equilibrated for 60 min. The strip of gall bladder was loaded with 0.5 g and the urinary bladder, with 1 g.

Wet weight of the muscle was determined before it was suspended in the medium. Muscle gravity was regarded as 1. Then, muscle volume was calculated by dividing the muscle weight by the muscle gravity. The length of the muscle suspended with a load in the medium was measured. The cross-sectional area of the muscle was calculated by dividing the muscle volume by the muscle length. Finally, a contraction was expressed as the tension to unit of cross-sectional area (kg force/cm²).

The PSS employed was a modified Tyrode’s solution of the following composition (mM): NaCl, 136.8; KCl, 5.4; CaCl₂, 2.5; MgCl₂, 1.0; NaHCO₃, 11.9 and glucose, 5.5. Hyperosmotic 65.4 mM K solution (hyper 65 K solution) was prepared by adding an appropriate volume of 3 M KCl stock solution. Isosmotic 40 mM K solution (iso-40 K solution) or isosmotic 154.2 mM K (iso-154 K solution) was made by substituting an appropriate amount of Na with equimolar K in the above solution. Sucrose (100 mM) or NaCl (50 mM) was added to iso-154K solution hyperosmotically. Pyruvic acid (5.5 mM) was applied to the high K solution in which glucose was removed, and the solution was neutralized by KOH. These solutions were aerated with a 95% O₂-5% CO₂ gas mixture at 36±1°C, pH 7.2.

Rate of oxygen consumption: The mucous layer and superficial connective tissue were excised from the gall bladder. The urinary bladder preparation after removal of the Trigonum vesicae, muscle sphincter vesicae and ostium ureteris was cut in a comb-like shape. The preparation was incubated in PSS for approximately 120 min until the muscle became stabilized. Oxygen consumption was measured by a similar method as described in an earlier paper (8), using a Clark-type polarograph electrode (YSI) connected with a biological oxygen monitor (YSI model 53).

Results

Changes in muscle tension in various kinds of high K solutions: The urinary bladder showed a large phasic contraction followed by tonic one in hyper-65K solution. When iso-154K solution was applied to the muscle, tension gradually decreased to approximately 24% of that by hyper-65K solution (Fig. 1A). In application of hyper-65K solution to the gall bladder, contractile tension without a phasic phase reached maximum at 30 min
and maintained at a steady level during 120 min. In iso-154K solution, the tonic contraction gradually decreased to approximately 23% of that by hyper-65K solution to the gall bladder at 120 min (Fig. 1B).

The application of hyper-65K solution induced a tension averaging 2.33 kg force/cm² at 120 min, as shown in Table 1. The addition of pyruvic acid (5.5 mM) showed no effect on the tension. Iso-154K solution induced a small tonic contraction averaging 0.56 kg force/cm². The addition of pyruvic acid (5.5 mM) to iso-154K solution prevented the decrease of tension completely and a hyperosmotic addition of 50 mM NaCl or 100 mM sucrose prevented it by half.

The application of hyper-65K solution to the gall bladder induced a tension averaging 1.81 kg force/cm² at 120 min, as shown in Table 2. When pyruvic acid was added to the high K solution, the tension was not affected. In iso-154K solution, the muscle showed a tension of 0.42 kg force/cm², which was about one forth of that by hyper-65K solution. The application of pyruvic acid caused a slight increase in the decreased tension by iso-154K solution. The hyperosmotic addition of NaCl (50 mM) or sucrose (100 mM) to the iso-154K solution prevented the decrease of tension to 69 or 89%, respectively.

Changes in the rate of O₂ consumption in various high K solutions: In the urinary bladder or gall bladder, the rate of basic O₂ consumption measured for 10 min was 0.234 or 0.223 μmol/g/min, respectively (Table 1). The rate of oxygen consumption increased two or three times by the addition of hyper-65K solution and maintained a steady level, which was 0.633 or 0.468 μmol/g/min at 120 min in urinary bladder or gall bladder, respectively.

When iso-154K solution was applied to the urinary bladder or gall bladder, the rate of oxygen consumption showed a transient increase and gradually reduced to 0.169 or 0.136 μmol/g/min at 120 min, respectively. These values were approximately 30% of that by hyper-65K solution. In the urinary bladder, the addition of pyruvic acid (5.5 mM) to the iso-154K solution prevented the decrease of O₂ consumption almost completely, and a hyperosmotic addition of 50 mM NaCl prevented it by 68%. However, hyperosmotic addition of 100 mM sucrose prevented it by half (Table 1). In the gall bladder, the application of pyruvic acid (5.5 mM) and a hyperosmotic addition of NaCl (50 mM) or sucrose (100 mM) prevented the decrease of the rate of oxygen consumption by 51, 42 or 36%, as shown in Table 2.

![Fig. 1. Changes in tension of guinea-pig urinary bladder (A) and gall bladder (B) in hyper-65K or iso-154K solution.](image)

| Condition               | kg force/cm² | μmol/g/min |
|-------------------------|--------------|------------|
| 1 Control               | —            | 0.234±0.005 (6) |
| 2 Iso-40K               | 1.37±0.12 (5) | 0.389±0.018 (4) |
| 3 Hyper-65K             | 2.33±0.13 (6) | 0.633±0.025 (11) |
| 4 +Pyruvic acid (5.5 mM)| 2.22±0.13 (5) | 0.653±0.035 (4) |
| 5 Iso-154K              | 0.56±0.04 (6) | 0.169±0.007 (6) |
| 6 +Pyruvic acid (5.5 mM)| 2.36±0.14 (5) | 0.608±0.039 (5) |
| 7 +NaCl (50 mM)         | 1.51±0.13 (5) | 0.432±0.041 (4) |
| 8 +Sucrose (100 mM)     | 1.13±0.08 (5) | 0.314±0.030 (4) |

All the values were obtained 120 min after the application of high K solution with various substances. Values of the mean±S.E.M. are shown, and numbers in parenthesis indicate number of experiments.
Table 2. Changes in tension and rate of oxygen consumption of gall bladder in various high K solutions

| Condition                        | kg force/cm² | µmol/g/min |
|----------------------------------|--------------|------------|
| 1 Control                        |              |            |
| 2 Iso-40K                         | 1.11±0.05    | 0.301±0.004 |
| 3 Hyper-65K                      | 1.81±0.27    | 0.468±0.041 |
| 4 +Pyruvic acid (5.5 mM)         | 2.09±0.15    | 0.390±0.041 |
| 5 Iso-154K                       | 0.42±0.06    | 0.136±0.006 |
| 6 +Pyruvic acid (5.5 mM)         | 0.66±0.07    | 0.237±0.044 |
| 7 +NaCl (50 mM)                  | 1.25±0.11    | 0.197±0.037 |
| 8 +Sucrose (100 mM)              | 1.60±0.07    | 0.168±0.023 |

All the values were obtained 120 min after the application of high K solution with various substances. Values of the mean±S.E.M. are shown, and numbers in parenthesis indicate number of experiments.

Fig. 2. Correlation between rate of oxygen consumption and tension of urinary bladder (A) and gall bladder (B) at 120 min in various high K solutions and normal physiological salt solution (PSS). Values and numbers on each of the points refer to those in Tables 1 and 2. The regression line drawn from the mean values fits Y=0.206X+0.133, r=0.950 (A) and Y=0.130X+0.161, r=0.875 (B), where Y is the rate of oxygen consumption, X is the tension and r is the coefficient. In line (B), values of No. 7 and 8 were not included in the calculation.

Figure 2A, plotted from values in Table 1, showed a close correlation (r=0.950) between muscle tension and rate of O₂ consumption at 120 min in the urinary bladder under various conditions. The correlation was described as follows: Rate of oxygen consumption (µmol/g/min)=0.206×muscle tension (kg force/cm²)+0.133. In the gall bladder, a correlation (r=0.875) was found between muscle tension and O₂ consumption without the values of hyperosmotic addition of NaCl (50 mM) and sucrose (100 mM), and it was described as follows: Rate of oxygen consumption (µmol/g/min)=0.130×muscle tension (kg force/cm²)+0.161 (Fig. 2B). The rate of oxygen consumption in both the muscles was shown to be linearly related to the developed tension in high K solution with various substances.

Discussion

It was reported that the rate of oxygen consumption of guinea-pig taenia coli in PSS was 0.4–0.6 µmol/g/min (1, 7, 9, 10), and that of vascular muscle was 0.07–0.08 µmol/g/min (7, 11). In urinary bladder or
gall bladder, $O_2$ consumption in PSS was 0.234 or 0.223 $\mu$mol/g/min, respectively. These values existed between the value of guinea-pig taenia coli and that of vascular smooth muscle.

It is known that various kinds of high K solutions increased the rate of oxygen consumption in many types of smooth muscles (12). In this experiment, hyper-65K solution increased $O_2$ consumption two or three times during contraction in the urinary bladder or gall bladder. On the other hand, the application of iso-154K solution to the urinary bladder and the gall bladder gradually decreased the tonic contraction and made the rate of oxygen consumption a transient increase followed by a small one. These data were consistent with the results in guinea-pig taenia coli (7, 10) and those of rabbit aorta (5) and porcine carotid artery (11).

In the urinary bladder, the application of pyruvic acid or NaCl prevented the inhibitions of tonic contraction and extra $O_2$ consumption by iso-154K solution. Further, it was reported that iso-154K solution did not change the relative cell water content of the urinary bladder calculated from tissue wet weight, dry weight and extracellular space (6). These results seem to confirm the proposal that glucose utilization of the urinary bladder as well as taenia coli is probably inhibited by Na deficiency in the medium (6). However, it was reported that the sugar transport in the smooth muscle of the detrusor of rat urinary bladder was increased by a decreased Na$^+$ and K$^+$ gradient which was induced by an addition of ouabain to or K depletion from normal medium (13). Nevertheless, a Na$^+$ and K$^+$ gradient in the muscle is thought to be extremely decreased by changing external Na$^+$ and K$^+$ concentrations in the present experiment; the iso-154K solution decreased the oxygen consumption in the urinary bladder suggesting the inhibition of sugar transport. This discrepancy may be caused by the different conditions and materials in both the experiments.

In the gall bladder, the hyperosmotic addition of 100 mM sucrose prevented the inhibition of tonic contraction by iso-154K solution, but had slight effect on decrease of $O_2$ consumption. The application of pyruvic acid was not very effective for tension and $O_2$ consumption. In the previous paper (6), iso-154K solution was reported to induce swelling of the gall bladder which was almost completely prevented by 100 mM sucrose. Accordingly, the inhibition of tonic contraction by iso-154K solution in gall bladder is probably caused mainly by cell swelling similar to that in rabbit aorta (5, 7) and partially by inhibition of glucose-Na symport.

It has been reported that the tension of smooth muscle closely correlated with the oxygen consumption (1, 7, 9, 10, 14–16). The urinary bladder consumed 0.206 $\mu$mol/g/min to maintain 1 kg force/cm$^2$ during high K-induced tonic contraction. The value of the urinary bladder is large and similar to that of taenia coli (0.218 $\mu$mol/g/min/kg force/cm$^2$), which was corrected from 0.33 $\mu$mol/g/min/kg force/cm$^2$ (7) by a different calculation for the cross-sectional area of the muscle, as described in “Methods”. The value of gall bladder is small (0.130 $\mu$mol/g/min/kg force/cm$^2$) and almost similar to that of porcine carotid artery (0.093 $\mu$mol/g/min/kg force/cm$^2$) (11). Addition of pyruvic acid to the urinary bladder treated with iso-154K solution was much more effective on the recovery of tension and oxygen consumption than those in the gall bladder as shown in Fig. 2A and B and Table 1. These results will support the concept that the glucose utilization of the urinary bladder during the hyper-65K-induced contraction was larger than that of the gall bladder.

In conclusion, the high K, Na-deficient solution inhibited the tonic contraction and extra $O_2$ consumption in the urinary bladder or gall bladder of guinea-pig. In the urinary bladder, the inhibition is probably caused by inhibition of Na-glucose symport, which is similar to that in taenia coli. However, the inhibition in the gall bladder seems to be mainly caused by cell swelling, similar to that of vascular smooth muscle, but partially caused by inhibition of the glucose symport.

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