A Comparative Study on the Contraction Induced by high K^+ / Na^+ Deficient Solution in Rat Uterus or Urinary Bladder

Junji KAMIZAKI, Kazumasa SHIMIZU, Shinjiro NAKAJYO
and Norimoto URAKAWA

Department of Veterinary Pharmacology, Nippon Veterinary and Zootechnical College,
Musashino, Tokyo 180, Japan

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Abstract—In the present paper, effects of high K^+ / Na^+ deficient solution on the rat uterus were examined on the mechanical response, wet weight of the tissue or rate of oxygen consumption, and they were compared with those in rat urinary bladder. In the uterus, isosmotic substitution of K^+ for Na^+ in a physiological salt solution (PSS) induced a contraction followed by a small sustained contraction, while hyperosmotic addition of KCl to PSS induced a sustained contraction. The hyperosmotic KCl addition increased the rate of oxygen consumption in comparison with that in PSS, and the substituted high K^+ / Na^+ deficient solution decreased it as compared with that in the hyperosmotic KCl addition. Similar results were shown in the urinary bladder. At 120 min after application of the substituted high K^+ / Na^+ deficient solution, the relative wet weight increased in the uterus, but did not change in the urinary bladder. Both the decrease in the developed tension and the increase in the wet weight of the uterus were prevented by the hyperosmotic addition of sucrose. In the urinary bladder, the decreased tension was significantly prevented by the hyperosmotic addition of NaCl to the PSS or substitution of pyruvate or oxalacetate for glucose, whereas it was slightly prevented by the hyperosmotic addition of sucrose. From these results, it is suggested that the decrease of the developed tension in isosmotically substituted high K^+ / Na^+ deficient solution in rat uterus is probably due to cell swelling and that the inhibition of contraction in urinary bladder is mainly caused by the inhibition of glucose utilization by Na^+ deficiency in the medium.

It is known that an elevation of potassium concentration (high K^+) in a physiological salt solution (PSS) induces a contraction in various kinds of smooth muscle preparations. It has been accepted that an application of high K^+ solution to smooth muscle causes depolarization accompanying a contraction which depends on extracellular Ca^{2+} entering through a voltage-dependent Ca^{2+} channel (1, 2). Accordingly, high K^+ is commonly used as a non-selective stimulant, a depolarizer and a pharmacological tool to open a voltage-dependent Ca^{2+} channel in smooth muscles. Therefore, the high K^+ -induced contraction has been widely employed for studies on excitation-contraction coupling in smooth muscles and also utilized as a standard con-

traction for the pharmacological analysis of chemical agents in various smooth muscles.

High K^+ solution is usually prepared either by adding KCl hyperosmotically to PSS or by substituting NaCl with isosmolar KCl (3). Although, a hyperosmotically added high K^+ solution induces a transient contraction followed by a large and sustained contraction, an isosmotically substituted high K^+ / Na^+ deficient solution induces a contraction followed by a gradual relaxation in various kinds of smooth muscles, e.g. guinea-pig taenia coli (4), trachea (5), urinary bladder (5), gall bladder (5), rabbit aorta (6) and trachea (7). These results were mainly obtained from the smooth muscle preparations of the guinea-pig or rabbit; however,

the uteri of these animals have not been examined as yet. As the uteri of guinea-pigs and rabbits show irregular estrus cycles and large spontaneous contractions, it is difficult to obtain a suitable myometrial preparation for the experiment.

In the present experiments, we examined the effects of isosmotically substituted high K^+/Na^+ deficient solution on mechanical response, wet weight of tissue and rate of oxygen consumption in the smooth muscle of the uterus of the rat, which shows a regular estrus cycle, and the results were compared with those of rat urinary bladder.

Materials and Methods

Virgin Wistar rats (250–300 g) in natural estrus were used. The stage of the estrus cycle was determined by microscopic examination of the vaginal smear, and then rats in the estrus stage were stunned by a blow on the head and bled to death. The uterus was removed from the opened abdomen by cutting at the cervical end. To obtain the myometrial preparation, a glass rod was inserted in the lumen. A thin layer of myometrium, 7–10 mm wide and 2–3 cm long, was carefully removed from the cervical end of the uterine horn with fine forceps, in a manner similar to how the longitudinal muscle layer is removed from an ileum (8).

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 preparation. The size of the preparation was 2–3 mm wide and 8–10 mm long. The mucous layer and superficial connective tissue were removed (9).

One end of the strips was bound to a glass

holder with a silk thread, and the other end was connected to a strain-gauge transducer (Nihon Kohden) with a thread. The muscles were suspended in an organ bath containing 15 ml of PSS and equilibrated for 60 min. The muscle contractions were isometrically recorded. The uterine strip was loaded with 0.5 g, and the urinary bladder was loaded with 3.0 g. After the 60 min incubation in PSS, 60 mM KCl was hyperosmotically applied twice to the muscle. When the muscle maintained a steady tonus after this treatment, various concentrations of KCl were applied. The tension developed by hyperosmotically added 65.4 mM KCl (hyper-65 K^+) solution at 120 min was referred to as the control (100%).

The PSS employed was a modified Tyrode's solution of the following composition: 136.8 mM NaCl, 5.4 mM KCl, 2.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, 11.9 mM $NaHCO_3$ and 5.5 mM glucose. A hyperosmotic high K^+ solution was prepared by adding appropriate volume of 3 M KCl stock solution. A substituted high K^+ solution was prepared by adding KCl and eliminating equimolar NaCl to maintain isosmolarity. In all substituted high K^+ solutions, 11.9 mM $NaHCO_3$ was substituted with equimolar $KHCO_3$. 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 (pH 7.2).

For determining the wet weight of a tissue, muscle strips were treated with various test solutions after the equilibration in PSS. During the incubation, the strips were removed from the organ bath at the appropriate time, blotted on a filter paper to exclude adhering solution and weighed with a balance. The ratio of cellular water content was calculated by the following equation:

$$\begin{aligned} \text{Ratio of cellular water content} = & \frac{\text{Wet weight (treated muscle) (mg)}}{\text{Wet weight (control muscle) (mg)}} \\ & \times \frac{\{1 - (\text{relative ECS} + \text{relative dry weight})\} (\text{treated muscle})}{\{1 - (\text{relative ECS} + \text{relative dry weight})\} (\text{control muscle})} \end{aligned}$$

Assuming that the specific gravity of the muscle is 1, the relative extracellular space (ECS) was expressed as ECS (mg)/wet

weight (mg). The relative dry weight was expressed as dry weight (mg)/wet weight (mg). Since ECS and the dry weight of the

rat uterus or urinary bladder were not determined in the present experiments, values reported in the literature were used in the calculation: the values of ECS in the guinea-pig uterus (10) and urinary bladder (5) have been reported to be 0.54 and 0.33, respectively. Furthermore, data on the several types of smooth muscle (5–7, 11, 12) indicated that the high K⁺/Na⁺ deficient solution did not change the relative value of the dry weight (0.17) of these tissues, but decreased the relative values of ECS by 20%. Accordingly, in this experiment, the ratio of cellular water content can be calculated by the above equation assuming that the relative dry weight of both the muscles is 0.17 in the normal PSS or the high K⁺/Na⁺ deficient solution, and that the relative values of ECS of the uterus and urinary bladder in normal PSS are 0.54 or 0.33, respectively, and the ECS values of both the muscles in the high K⁺/Na⁺ deficient solution are decreased by 20% (0.43 for the uterus and 0.26 for the urinary bladder).

Oxygen consumption was measured by a method similar to that described in the paper by Stephens et al. (13), using a Clark-type polarograph electrode (YSI) connected to a biological oxygen monitor (YSI model 53).

Values were expressed as the mean $\pm$ S.E.M., and statistical analyses were performed by Student's *t*-test.

Results

Changes in muscle tension and wet weight of tissue

Uterus: When hyper-65K⁺ solution was applied to the uterus, the muscle gradually increased its tension which reached maximum at 30 min and maintained a steady level during 120 min. Figure 1A shows the statistical results of the time course of tension changes. Isosmotic 60 mM or 120 mM KCl (iso-60K⁺ or iso-120K⁺) solution induced a phasic contraction followed by a tonic contraction which maintained a steady level and reached 85.9% or 45.9% of that by hyper-65K⁺ solution at 120 min, respectively. Iso-154K⁺ solution also induced a phasic contraction, then slowly increased the muscle tension which reached maximum at 15 min and gradually attenuated to 12.5% at 120 min.

Since these high K⁺-induced contractions in the uterus were not affected by the treatment with 10⁻⁷ g/ml tetrodotoxin, an inhibitor of nerve conduction, or 10⁻⁶ M atropine, an anticholinergic drug, it seems that the contractions are not mediated by released nerve transmitters.

While hyper-65K⁺ and iso-60K⁺ solutions significantly decreased the relative value for the wet weight of the tissue at 120 min and iso-120K⁺ solution did not affect it, iso-154K⁺ solution showed a remarkable increase in the wet weight of tissue (Fig. 2A). The relative cellular water content of the uterus was estimated to be 1.55 in the iso-154K⁺ solution (Table 1), and the iso-154K⁺ solution seemed to cause the uterine cells to swell.

The concentration-response relationship between K⁺ concentration and muscle tension or relative wet weight of the tissue is shown in Fig. 3A. The curve of muscle tension is bell-shaped and that of the wet weight of the tissue is almost flat.

Urinary bladder: When hyper-65K⁺, iso-60K⁺ or iso-120K⁺ solution was applied to the tissue, the muscle transiently increased its tension, then kept a steady level. The levels in the latter two solutions were 106.0% and 124.2% of that by hyper-65K⁺ solution at 120 min, respectively. An application of iso-154K⁺ solution to the tissue induced a large and phasic contraction followed by gradual attenuation to 6.4% at 120 min (Fig. 1B). These high K⁺-induced contractions were not affected by the treatment with tetrodotoxin and atropine.

Although hyper-65K⁺ solution markedly decreased the wet weight of the urinary bladder, the other solutions decreased it only slightly (Fig. 2B). The relative cellular water content in the iso-154K⁺ solution was estimated to be 0.98 (Table 1). The concentration-response curve for muscle tension of the urinary bladder, which was bell-shaped, was different from that of the uterus, which had a single peak at 60 mM K⁺, and the curve for relative wet weight in the urinary bladder was almost flat and showed a smaller change than that in the uterus (Fig. 3B).

Hyperosmotic addition of sucrose

In the uterus, the addition of sucrose at a concentration of 25, 50 or 100 mM to the

Fig. 1. Changes in tension of rat uterus (A) and urinary bladder (B) in high K⁺ solutions. 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%), and the mean $\pm$ S.E.M. of six experiments is shown. In some cases, the S.E.M. was smaller than the size of the symbol.

medium reversed the inhibition of muscle tension by the iso-154K⁺ solution to 88.3, 90.3 or 48.1% of that by hyper-65K⁺ solution at 120 min, respectively. The concentration-response relationship between sucrose concentration and muscle tension or wet weight of the tissue in iso-154K⁺ solution is shown in Fig. 4A. Increasing sucrose concentration induced an increase in the tension, with the exception of 100 mM sucrose addition, and

it decreased the relative wet weight.

In the urinary bladder, the decreased tension induced by the iso-154K⁺ solution was slightly increased by the hyperosmotic addition of sucrose at a concentration of 25 or 50 mM (Fig. 4B). Even the addition of 100 mM sucrose to the urinary bladder prevented the decrease in tension only to 50.4% of that by hyper-65K⁺ solution, and the prevention was almost similar to that in the uterus. The

Fig. 2. Changes in relative wet weight of rat uterus (A) and urinary bladder (B) in high K⁺ solutions. Normal Tyrode's solution (PSS), hyper-65K⁺, iso-60K⁺, iso-120K⁺ or iso-154K⁺ was applied at 0 min. Relative wet weight is expressed as wet weight (mg)/control wet weight (mg), and the mean $\pm$ S.E.M. of six experiments is shown. In some cases, the S.E.M. was smaller than the size of the symbol.

relative wet weight of the urinary bladder was slightly decreased by increasing sucrose concentration.

In these results, the hyperosmotic addition of sucrose probably confirms the correlation-ship between tension and relative wet weight, and the correlation in the uterus was higher than that in the urinary bladder.

Effects of additions of NaCl and use of pyruvate or oxalacetate on the contraction induced by iso-154K⁺ solution

The developed tension of the uterus in iso-154K⁺ medium decreased to 12.5% of that in hyper-65K⁺ medium at 120 min. A hyperosmotic addition of NaCl (25 mM) to the iso-154K⁺ medium prevented the decrease of tension to 65.2% and the increase of wet weight. The substitution of pyruvate (5.5 mM) or oxalacetate (5.5 mM) for glucose in iso-154K⁺ solution did not affect the decreased tension (Table 2).

In the urinary bladder, a hyperosmotic

Fig. 3. Changes in muscle tension (●) and relative wet weight (▲) obtained at 120 min after the application of substituted high K⁺ solutions. Muscle tension induced by hyper-65K⁺ solution at 120 min was the control (100%). Relative wet weight is expressed as wet weight (mg)/control wet weight (mg), and the mean±S.E.M. of six experiments is shown. In some cases, the S.E.M. was smaller than the size of the symbol. A: uterus, B: urinary bladder.

Table 1. Changes in relative wet weight and relative cellular water content in uterus and urinary bladder of rat in iso-154K⁺ solution

			Uterus	Urinary bladder
Iso-154K ⁺	Wet weight		1.12±0.02 (6)	0.98±0.03 (6)
	(Cellular water content)		(1.55)	(0.98)
+Sucrose (50 mM)	Wet weight		0.91±0.01 (6)	0.84±0.01 (5)
	(Cellular water content)		(1.26)	(0.84)
(100 mM)	Wet weight		0.89±0.01 (6)	0.83±0.02 (6)
	(Cellular water content)		(1.23)	(0.83)
+NaCl (50 mM)	Wet weight		0.92±0.01 (6)	0.89±0.01 (4)
	(Cellular water content)		(1.27)	(0.89)

Values of the mean±S.E.M. are shown, and numbers in parenthesis indicate number of experiments. The calculation of cellular water content was described in "Materials and Methods".

Fig. 4. 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. Muscle tension induced by hyper-65K⁺ solution at 120 min was the control (100%). Relative wet weight is expressed as wet weight (mg)/control wet weight (mg), and the mean $\pm$ S.E.M. of five or six experiments is shown. In some cases, the S.E.M. was smaller than the size of the symbol. A: uterus, B: urinary bladder.

addition of NaCl (25 mM) to the iso-154K⁺ solution reversed the decrease of tension to 110.3%. It was also revealed that the addition of NaCl was more effective than that of sucrose. The addition of pyruvate (2.75 mM) or oxalacetate (2.75 mM) to iso-154K⁺ solution without glucose prevented satisfactorily the decrease in tension by iso-154K⁺ solution (Table 2).

Changes in the rate of oxygen consumption in various high K⁺ solutions

In the uterus, the rate of basic oxygen consumption measured for 10 min was 0.481

$\mu\text{mol/g/min}$ (Table 3). The rate of oxygen consumption was increased approximately 1.4 times by the addition of hyper-65K⁺ solution and maintained a steady level which was 0.674 $\mu\text{mol/g/min}$ at 120 min. The addition of the iso-154K⁺ solution decreased the rate of oxygen consumption to 0.400 $\mu\text{mol/g/min}$ in comparison with that of hyper-65K⁺ solution. The application of pyruvate (5.5 mM) to the iso-154K⁺ solution without glucose did not affect the decrease of oxygen consumption.

In the urinary bladder, the rate of basic

Table 2. Effects of sucrose, NaCl, pyruvate or oxalacetate on iso-154 K⁺-induced contraction in uterus and urinary bladder of rat

		Uterus	Urinary bladder
Hyper-65K ⁺		100	100
Iso-154K ⁺		12.5±3.1 (6)	6.4±1.8 (6)
+ Sucrose	(50 mM)	90.3±3.9 (6)***	33.2±4.5 (6)***
+ NaCl	(25 mM)	65.2±9.2 (4)***	110.3±8.6 (4)***
+ Pyruvate	(5.5 mM)	17.9±4.7 (7)	—
	(2.75 mM)	—	101.0±11.0 (6)***
+ Oxalacetate	(5.5 mM)	18.7±6.3 (7)	—
	(2.75 mM)	—	111.7±11.9 (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%). Values of the mean±S.E.M. are shown, and numbers in parenthesis indicate number of experiments. ***P<0.001 (Significantly different from iso-154K⁺).

Table 3. Changes in the rate of oxygen consumption (μmol/g/min) of uterus and urinary bladder of rat in various high K⁺ solutions

	Uterus	Urinary bladder
Control	0.481±0.030 (7)	0.325±0.017 (12)
Hyper-65K ⁺	0.674±0.067 (4)*	0.596±0.042 (4)**
Iso-154K ⁺	0.400±0.031 (7)##	0.260±0.028 (6)##
+ Pyruvate (5.5 mM)	0.435±0.029 (4)	—
+ Pyruvate (2.75 mM)	—	0.362±0.017 (8)††

All the values were obtained 120 min after the application of high K⁺ solution with substance. Pyruvate was simultaneously applied with the glucose-free high K⁺ solution. Values of the mean±S.E.M. are shown, and numbers in parenthesis indicate number of experiments. *P<0.05, **P<0.01 (Significantly different from the control). ##P<0.01 (Significantly different from hyper-65K⁺). ††P<0.01 (Significantly different from iso-154K⁺).

oxygen consumption was 0.325 μmol/g/min (Table 3), and increased by the addition of hyper-65K⁺ solution to 0.596 μmol/g/min at 120 min. The addition of the iso-154K⁺ solution decreased the rate of oxygen consumption to 0.260 μmol/g/min. However, the application of pyruvate (2.75 mM) to iso-154K⁺ solution prevented the decrease of the rate of oxygen consumption.

Discussion

In the rat uterus, iso-154K⁺ solution rapidly induced a maximum contraction, then gradually decreased the muscle tension, and it remarkably increased the relative wet weight or cellular water content. This decrease in the developed tension was prevented by the hyperosmotic addition of sucrose or NaCl, but not affected by the substitution of pyruvate or oxalacetate in iso-154K⁺ solution

for glucose. Moreover, the hyperosmotic addition of sucrose or NaCl decreased the increased wet weight. The decreased oxygen consumption was not affected by the addition of pyruvate in iso-154K⁺ solution. Jones et al. (11) observed that during a 30 min incubation in substituted high K⁺/Na⁺ deficient solution the wet weight of rabbit aorta did not change and ECS significantly decreased, resulting in an increase in cellular water content. They also noted that the swelling of the cell was prevented by the addition of sucrose or by using a K⁺ salt with an impermeable anion, SO₄²⁻. On the other hand, Suzuki et al. (6) extended the observation period in the aorta and found that the wet weight increased after a 60 min incubation with substituted high K⁺/Na⁺ deficient solution and the cellular water content significantly increased. They have suggested

that there is a close relationship between cell swelling and inhibition of contraction in the aorta treated with KCl solution. Therefore, the decreased tension induced by isosmotically substituted high K⁺/Na⁺ deficient solution in the uterus is also due probably to cell swelling. Although the developed tension by the hyperosmotic addition of 100 mM sucrose was decreased to about half that by 50 mM sucrose, it is probably due to inhibition of an excitation-contraction coupling by an excess hyperosmolarity (14).

On the other hand, in the urinary bladder, iso-154K⁺ solution decreased the tension, but did not change the relative wet weight. The decrease of contraction induced by iso-154K⁺ solution was significantly reversed by the application of pyruvate or oxalacetate, and the hyperosmotic addition of NaCl to the iso-154K⁺ solution was more effective than that of sucrose. The decrease of oxygen consumption was prevented by the addition of pyruvate in iso-154K⁺ solution. It is well-known that the tonic contraction induced by hyperosmotic KCl solution in guinea-pig taenia coli is dependent on the external glucose (15, 16). Both pyruvate and oxalacetate are as effective as glucose on the contraction (16). However, glucose is not utilized in the absence of external Na⁺, while pyruvate or oxalacetate is utilized even in Na⁺ deficient solution (17). From these results, it has been suggested that the transport of glucose across the cell membrane is directly dependent on extracellular Na⁺ (17, 18). Thus, the inhibition of contraction induced by the high K⁺/Na⁺ deficient solution in taenia coli is due to inhibition of an utilization of glucose resulting from Na⁺ deficiency and partly to the swelling of the muscle. However, the inhibition of glucose utilization is solely the cause of the decreased tension of the guinea-pig urinary bladder in the high K⁺/Na⁺ deficient solution (5, 19). Therefore, the decreased tension induced by iso-154K⁺ solution in the rat urinary bladder as well as the guinea-pig urinary bladder can be explained mainly by the inhibition of the utilization of glucose which shows a co-symport with Na⁺ in the medium.

Shimizu et al. (5) have proposed that there are three types of inhibition by the high K⁺/

Na⁺ deficient solution: the inhibition is due (I) to a swelling of the cell, as in the case of rabbit aorta and guinea-pig trachea, (II) to an inhibition of utilization of glucose resulting from Na⁺ deficiency in the medium, as in guinea-pig urinary bladder, (III) to both the swelling and the inhibition of glucose utilization, as in rabbit trachea, guinea-pig gall bladder and taenia coli. By this classification, the inhibition in the rat uterus belongs to group I and that in rat urinary bladder to the group II.

In summary, it is suggested that the decrease of the developed tension in isosmotically substituted high K⁺/Na⁺ deficient solution in rat uterus is probably due to cell swelling and that the inhibition of contraction in urinary bladder is mainly caused by the inhibition of glucose utilization by Na⁺ deficiency in the medium.

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