

Effects of Levromakalim on Ureteral Peristaltic Function and Cystometrogram in Rats

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ABSTRACT—We studied the effect of levromakalim on the function of the urinary tract in rats. Using anesthetized rats, ureteral peristaltic movement of only the ureter region or the ureter with the kidney was induced by fluid infusion into the ureter lumen. Levromakalim (0.03 and 0.3 mg/kg, i.v.) exerted a stronger inhibitory effect on the peristaltic movement of the ureter region distant from the pelvis than on that near the pelvis, and the inhibitory effect of levromakalim (0.03 mg/kg, i.v.) was not antagonized by glibenclamide (0.1 mg/kg, i.v. or 10 mg/kg, i.p.). Topical application of levromakalim (injection volume, 0.1 ml; 10^{-4} or 10^{-3} M), which was injected via a vessel near the ureter inhibited ureteral peristaltic movement and the inhibitory effect levromakalim (10^{-4} M), was not antagonized by glibenclamide (10^{-3} M) injected via the same route. Levromakalim (0.3 mg/kg, i.v.) did not interrupt micturition in anesthetized and conscious rats. In conscious rats, the micturition interval was prolonged; and in anesthetized rats, the peak pressure during micturition was reduced. After injection of levromakalim (0.3 mg/kg, i.v.), vesicoureteral reflux did not occur. In the movements of the ureter, urinary bladder and urethra, levromakalim exerted the strongest inhibitory effect on the ureteral peristalsis.

Keywords: Levromakalim, Glibenclamide, Ureteral peristaltic function (rat), Cystometrogram, Micturition

Cromakalim, a K^+ channel opener, exerts relaxant effects in many kinds of smooth-muscle organs by hyperpolarizing the membrane through activation of K^+ channels (1–5), and their clinical usefulness for the treatment of urinary bladder dysfunctions in which the detrusor muscle becomes hyperexcitable is suggested from studies on guinea pigs and rats (4, 6), as well as hypertension (7) and asthma in patients (8). Recently, we reported that levromakalim (BRL 38227), which is the (–) enantiomer of cromakalim and has identical properties but can be used at a lower dose level (9), had a strong inhibitory effect on myogenic ureteral peristaltic movement when a solution containing the drug was infused into the ureter lumen in anesthetized rats (10). The lower urinary tract, including the ureter, urinary bladder and urethral proximal sphincter, is composed of smooth muscle; and its function of urine transport, storage or evacuation (micturition) is dependent upon the integrated motility of these organs in the lower urinary tract. Therefore, it is of interest to clarify the effect of levromakalim on the functions of the lower urinary tract. In the present study using rats, levromakalim was systemically or topically injected, and its

effects on ureteral peristaltic movement and the cystometrogram were investigated.

MATERIALS AND METHODS

For studying the effects of drugs on ureteral peristaltic movement, we used a method that was a partial modification of our system previously used to investigate the effects of drugs applied from the ureter lumen (10). The experimental setup is shown in Fig. 1. Male Sprague-Dawley rats (weighing 450–650 g) were anesthetized with urethane (0.75–1.0 g/kg, i.p.) and alpha-chloralose (19–25 mg/kg, i.p.) and placed in a supine position. In the abdomen, a midline incision extending from the urinary bladder to the kidney was made, and the left ureter was exposed. Two pieces of silicone tubing (outer diameter, 1.0 mm; inner diameter, 0.5 mm; about 20-cm-long) were prepared as kidney-side and bladder-side cannulas, and a needle (1/3) was attached to the end of each. For the generation of peristaltic movement, two methods were used. In one, ureteral peristaltic movement was generated only in the ureter region. The needle of the kidney-side

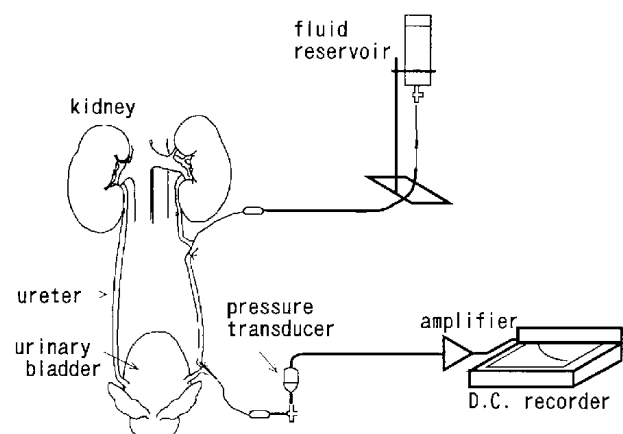


Fig. 1. Setup used for generation and measurement of peristaltic movement in the ureter region in anesthetized rats. When the peristaltic pressure signals of the ureter along with the kidney were measured, only the bladder side-cannula was inserted, and a small amount of fluid was injected into the ureter lumen through the cannula instead of infusion of fluid into the ureter lumen. For details, see Materials and Methods.

cannula was inserted into the ureter from the region near the kidney in the direction of the bladder, and the ureter was ligated around the needle. The needle of the bladder-side cannula was inserted into the ureter near the bladder in the direction of the kidney, and then the ureter was ligated. The distance between the two needle tips was at least 4 cm. As a fluid, Tyrode's solution lacking glucose was used, and it was infused into the ureter lumen from a reservoir through the kidney-side cannula at room temperature. The surface of the fluid in the reservoir was adjusted to a height of approximately 27 cm of fluid above the animal's back. In order to maintain the flow at a low and constant rate, it was passed through a thin polyethylene tube (SP-8; Natsume, Tokyo), approximately 8-cm-long, and then allowed to flow into the kidney-side cannula. Fluid that had passed through the ureter lumen was drained out from the end of the bladder-side cannula. To

generate ureteral peristaltic movement and record the intraluminal pressure signals, the bladder-side cannula was connected to a three-way cock attached to a pressure transducer, and the cock was turned to allow fluid to flow from the bladder-side cannula into the transducer. Pressure signals induced by ureteral movement were measured for 30–40 min. Then the bladder-side cannula was disconnected from the cock, and the measurement was interrupted for about 30 min. These procedures were repeated in order to record the ureteral pressure signals and to allow a rest period for the ureter. The pressure signals were delivered by an amplifier (AP-601G; Nihon Kohden, Tokyo) and recorded by a D.C. recorder (Tokai Irika, Tokyo). For the other method, ureteral peristaltic movements were generated by distention of the ureter along with the kidney. In this case, only the bladder-side cannula was inserted into the ureter. A small amount of fluid, less than 0.03 ml, was injected into the ureter lumen through the cannula, and the pressure signals were obtained and recorded in the same way as described above.

When a drug was applied systemically, it was injected through a femoral vein or intraperitoneally at a location where the drug-containing solution did not come into direct contact with the ureter. When a drug was applied topically into the ureter, the cannula for drug injection was inserted into the left internal spermatic vessel near the kidney. The drug was given in a volume of 0.1 ml and then flushed with 0.3 ml of vehicle. When only the effect of vehicle was investigated, 0.4 ml of vehicle was injected through the cannula. The abdomen of each rat was covered with tissue paper to prevent drying during the experiment.

The following parameters were calculated from the recorded traces of the peristaltic movement: 1) A, average amplitude of peristaltic pressure signals (cmH_2O); 2) N, the number of peristaltic pressure signals in 2 min.

Recording of cystometrograms in anesthetized and conscious rats was performed with male Wistar rats (weighing 250–350 g), using the methods described in our previ-

Table 1. Values of parameters of ureteric peristaltic movement when infusion of fluid into the ureter lumen was repeated ($n=5$)

	First time		Second time		3rd time		4th time		5th time		6th time	
	A	N	A	N	A	N	A	N	A	N	A	N
Mean	7.6	12.8	7.0	11.1	7.1	9.4	6.8	10.9	6.7	8.9	7.4	8.4
S.E.	1.2	1.3	1.1	1.9	1.1	1.3	1.0	2.2	0.9	1.8	1.4	1.8

A cannula was inserted into the ureter near the kidney, and through the cannula, Tyrode's solution was infused into the ureter lumen from a reservoir, permitting ureteral distention to a fixed intraluminal pressure (approximately $27 \text{ cmH}_2\text{O}$). Another cannula was inserted into the ureter near the bladder, and the ureteral intraluminal pressure was recorded through the cannula for about 60 min. Fluid was drained out via the cannula inserted near the bladder, and the pressure recording was discontinued for about 60 min. This procedure was repeated six times. The parameters A and N were calculated from tracings of the pressure signals. A: amplitude (cmH_2O) of the ureteral peristaltic pressure. N: number of pressure signals of ureteral peristaltic movement in 2 min.

ous papers (11, 12). For i.v.-injection in conscious rats, an i.v.-injection catheter was inserted through the femoral vein. As each rat in a Ballman cage moved occasionally during the experiment, a lethal dose of pentobarbital was injected intravenously through the cannula after the experiment, and its retention in the vein was confirmed. The amount of solution excreted from the urethra in conscious rats was measured by using a F.D. pickup (TB-611T, Nihon Kohden).

In anesthetized rats, contrast medium was infused continuously into the bladder, and the ureterogram was taken using soft X-ray fluoroscopy (Softex, Tokyo) at 60 and 90 min after injection of the drug.

Each anesthetized rat was warmed with a lamp, and its temperature (adjusted close to 35°C was measured with a thermometer inserted into the mouth. Each conscious rat was warmed arbitrarily with a lamp.

Experimental values are expressed as the mean \pm S.E. The significance of differences was analyzed by Student's *t*-test at $P < 0.05$.

Drugs used were levcromakalim ((-)-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl)-2H-1-benzopyran-3-ol; a gift from Smith-Kline Beecham, Tokyo), glibenclamide (a gift from Yamanouchi Pharmaceutical Co., Ltd., Tokyo) and nifedipine (Sigma, St. Louis, MO, USA). Glibenclamide and levcromakalim for i.v. injection were dissolved in pure ethanol or 70% ethanol solution, respectively, to give a 10^{-2} M stock solution. The stock solution was diluted with saline before use. Glibenclamide and nifedipine for i.p. injection were dissolved in polyethylene glycol. Urografin 60® (Nihon Schering, Osaka) was used as a contrast medium. The concentration of each drug was examined in at least four different rats.

RESULTS

The effects of intravenously or intraperitoneally injected drug on ureteral peristaltic pressure movement that was induced by infusion of fluid through the kidney-side cannula

Table 1 shows the parameters of the pressure signals induced by ureteral peristaltic movement in response to infusion of fluid through the kidney-side cannula repeated at about 30-min intervals. There was no significant difference between the parameters of the first trace and those of the other traces.

The effects of levcromakalim on ureteral peristaltic movement are shown in Fig. 2A and summarized in Table 2. Injection of 7% ethanol solution (1 ml/kg, i.v.), which was used for the vehicle of levcromakalim (0.3 mg/ml), did not affect the frequency and amplitude of the peristaltic pressure signals. After injection of levcromakalim (0.03 mg/kg, i.v.), peristaltic pressure signals disap-

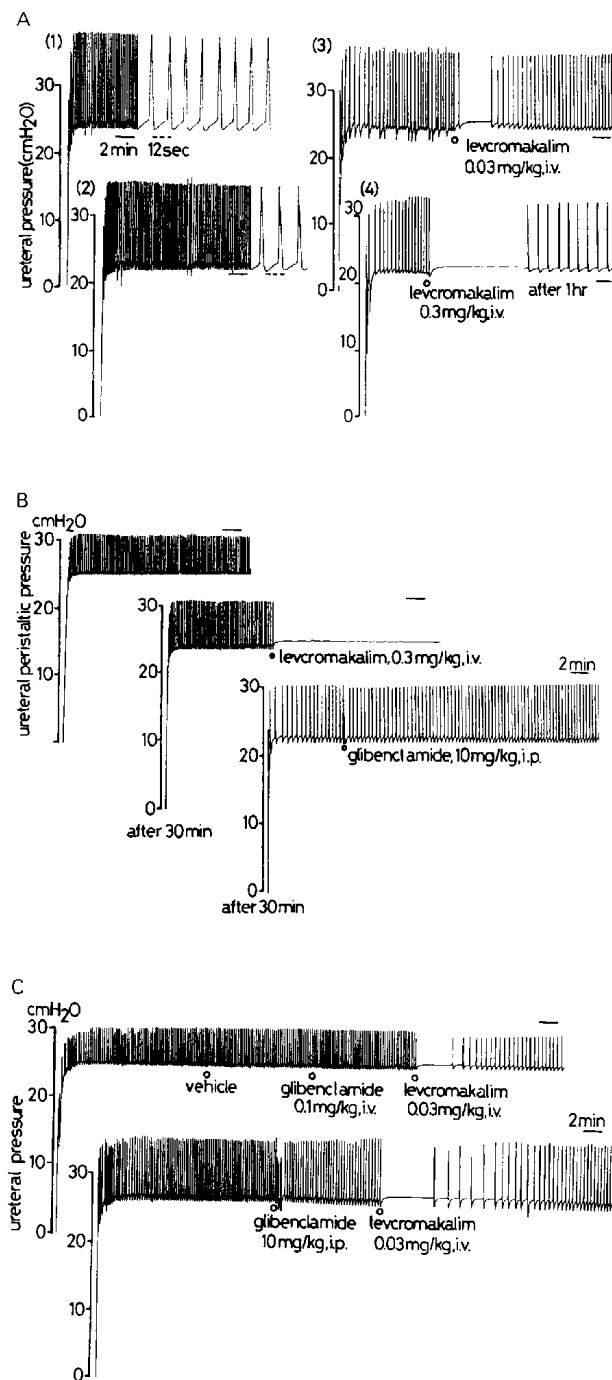


Fig. 2. Effects of levcromakalim (A), effects of glibenclamide after application of levcromakalim (B) and effects of levcromakalim after application of glibenclamide (C) on ureteral peristaltic pressure signals in an anesthetized rat. The ureteral peristaltic pressure signals were induced by infusion of fluid through the kidney-side cannula. For details, see Fig. 1. Vertical bar: intraluminal ureteral pressure (cmH₂O). Horizontal bars: 2 min (solid line) and 12 sec (broken line). Recordings of A and B were performed in a different rat, and the number in parentheses above each trace indicates the order of the experiment. Between recordings of peristaltic pressure signals, the bladder-side cannula was disconnected from the transducer for at least 30 min. Each record of C was obtained from a different rat.

Table 2. Effects of levromakalim and nifedipine on ureteric peristaltic movement

i.v. injection of ethanol or levromakalim (n=6)														
	Before injection		Vehicle (7% ethanol)		Before injection		0.03 mg/kg		120 min		0.3 mg/kg		120 min	
	A	N	A	N	A	N	A	N	A	N	A	N	A	N
Mean	10.5	14.2	10.5	14.0	9.8	12.6	9.8	9.4*	8.3	8.7	0	0	9.2	2.0*
S.E.	1.5	3.3	1.4	3.3	1.3	3.6	1.3	3.3	1.0	2.0	0	0	1.2	0.4

i.p. injection of nifedipine (n=5)											
	Before injection		3 mg/kg		60 min		10 mg/kg		120 min		
	A	N	A	N	A	N	A	N	A	N	
Mean	8.9	18.8	7.2	8.5*	6.2	10.4	3.3*	3.5*	5.4	7.7	
S.E.	1.0	4.4	0.4	1.3	0.3	1.5	1.1	1.8	0.4	1.1	

The experimental method is described in Table 1. The values for levromakalim were calculated from the pressure signals obtained just after injection of the agent, and those for nifedipine were calculated from the pressure signals 10–20 min after injection. Time (min) indicates time after injection of each dose of drug. A: amplitude (cmH₂O) of the ureteral peristaltic pressure, N: number of pressure signals of ureteral peristaltic movement in 2 min. *P < 0.05 represents a significant difference between the values before and after drug injection. Levromakalim (0.3 mg/kg, i.v.) and nifedipine (10 mg/kg, i.p.) were injected about 120 min and 60 min after injection at the lower dose, respectively; and the values for levromakalim (0.3 mg/kg, i.v.) and nifedipine (10 mg/kg, i.p.) were compared with those before injection at each respective dose.

peared for 2.1 ± 0.5 min (n=6). When levromakalim (0.3 mg/kg, i.v.) was added about 60 min after injection of levromakalim (0.03 mg/kg, i.v.), the pressure signals disappeared completely for 30 min in four out of six rats; and in another two rats, the pressure recovered 11 and 12 min after injection. When the ureteral peristaltic movement was abolished by levromakalim, the static pressure level was almost the level before the onset of the pressure raise. Although the peristaltic pressure resumed at about 60 min after the injection of levromakalim (0.3 mg/kg, i.v.), their frequency was very low. The decrease in the frequency of peristaltic movement 30 min after injection of levromakalim (0.3 mg/kg, i.v.) was not reversed by glibenclamide (10 mg/kg, i.p.) (Fig. 2B); and at about 10 min after application of glibenclamide (0.1 mg/kg, i.v. or 10 mg/kg, i.p.), levromakalim (0.03 mg/kg, i.v.) exerted almost the same inhibitory effect on the peristaltic movement as that observed without application of glibenclamide (Fig. 2C). Glibenclamide at the dose described above did not change the pattern of ureteral peristaltic movement. We investigated the effect of another type of smooth muscle relaxant, a Ca antagonist, on ureteral peristaltic movement and the time of appearance of the effect of i.p.-injected drug on the movement. Nifedipine at a dose of 3 mg/kg gradually decreased the frequency, and nifedipine at a dose of 10 mg/kg decreased both the frequency and amplitude of pressure signals within 10 min (Fig. 3). In addition to the inhibitory effects on peristaltic movement summarized in Table 2, nifedipine

at doses of 3 and 10 mg/kg elevated the basal pressure to 1.4 ± 0.4 and 0.8 ± 0.2 cmH₂O, respectively (n=5).

The effect of drugs that were topically applied in an injection volume of 0.1 ml are shown in Fig. 4 and summarized in Table 3. Levromakalim (10^{-4} M and 10^{-3} M) inhibited the peristaltic movement (Fig. 4A). Glibenclamide (10^{-3} M) that was topically applied in an equivalent injection volume neither changed the pattern of the peristaltic movement nor antagonized the inhibitory effect of levromakalim on the movement (Fig. 4B).

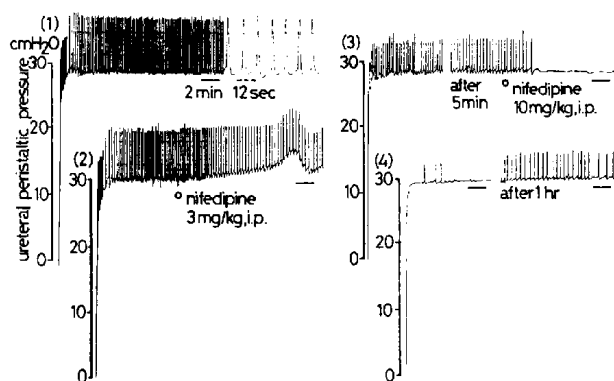


Fig. 3. Effects of nifedipine on ureteral peristaltic pressure signals in an anesthetized rat. The ureteral peristaltic pressure signals were induced by infusion of fluid through the kidney-side cannula. For details, see the legend of Fig. 2. Each record was obtained from the same rat.

Effects of drug on the ureteral peristaltic pressure signals recorded in the ureter along with the kidney

Figure 5 shows a trace of the peristaltic pressure signals of the ureter along with the kidney before and after ligation of the ureter near the kidney. Before ligation of the

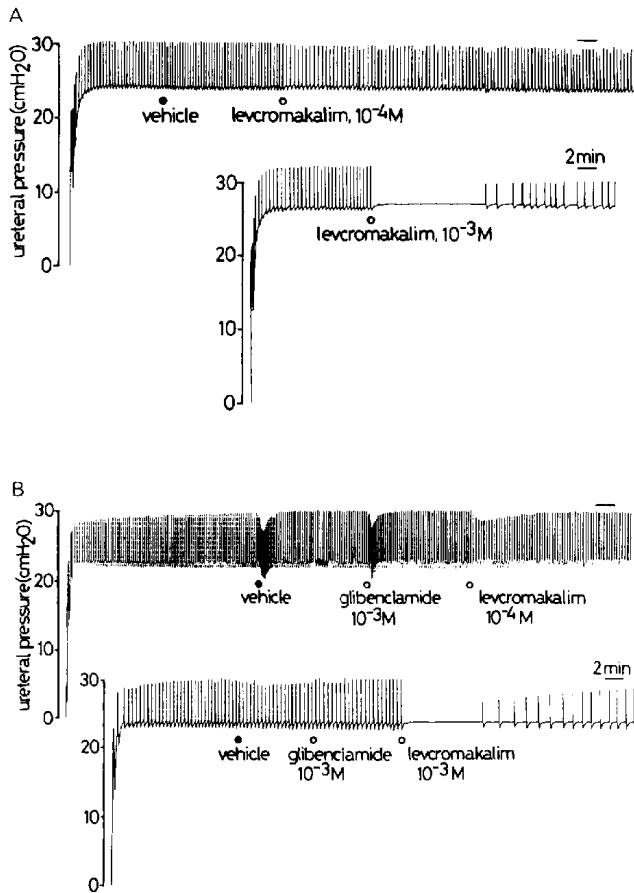


Fig. 4. Effects of topical application of levcromakalim before (A) and after (B) topical application of glibenclamide on ureteral peristaltic pressure signals in an anesthetized rat. The ureteral peristaltic pressure signals were induced by infusion of fluid through the kidney-side cannula. For details, see the legend of Fig. 2. Injected volume of drug solution was 0.1 ml, applied through the catheter inserted into a spermatic vessel near the kidney. Recordings of A and B were performed in a different rat.

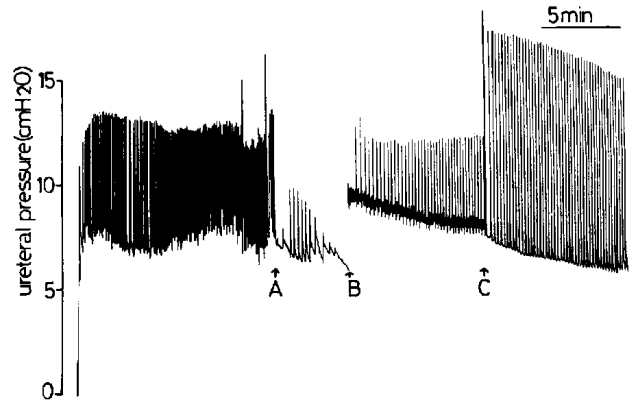


Fig. 5. Ureteral peristaltic pressure signals of the ureter along with the kidney before and after ligation of the ureter near the kidney in an anesthetized rat. The ureteral peristaltic pressure signals were induced by injection of a small amount of fluid through the cannula inserted into the ureter near the bladder. For details, see Fig. 1. At A, connective tissue around the ureter near the kidney was removed in order to pass the ligation thread under the ureter. At B, a small amount of fluid was applied to the ureter lumen through the cannula in order to raise the baseline pressure. At C, the ureter was ligated near the kidney. Vertical bar: intraluminal ureteral pressure (cmH₂O). Horizontal bar: 5 min.

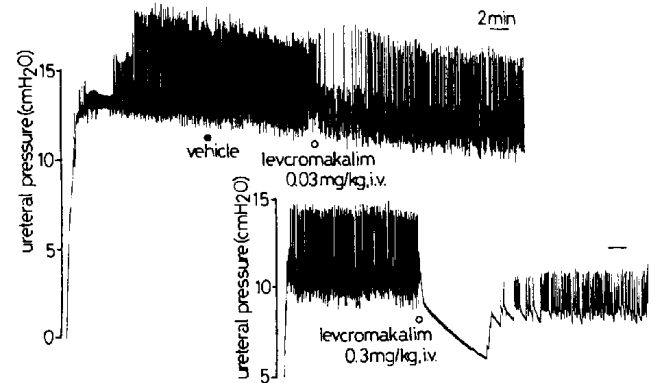


Fig. 6. Effect of levcromakalim on ureteral peristaltic pressure signals of the ureter along with the kidney in an anesthetized rat. The ureteral peristaltic pressure signals were induced by injection of a small amount of fluid through the cannula inserted into the ureter near the bladder. For details, see the legend of Fig. 5.

Table 3. Effects of levcromakalim on ureteric peristaltic movement when drugs were applied topically from the spermatic vessel (n=6)

	Before injection		Vehicle (7% ethanol)		Before injection		10 ⁻⁴ M		10 min		Before injection		10 ⁻³ M		30 min	
	A	N	A	N	A	N	A	N	A	N	A	N	A	N	A	N
Mean	9.7	10.2	9.7	10.7	9.0	8.6	7.8*	6.2*	9.0	7.4	9.3	7.9	0	0	6.8**	2.3**
S.E.	1.5	1.6	1.5	1.7	1.6	1.2	2.2	1.6	1.6	1.0	1.6	1.2	0	0	1.4	0.4

The experimental method is described in Table 1. Tyrode's solution was injected into the ureter lumen through the cannula inserted into the ureter near the kidney. Time (min) indicates time after injection of each concentration of drug. A: amplitude (cmH₂O) of the ureteral peristaltic pressure, N: number of pressure signals of ureteral peristaltic movement in 2 min. *P<0.05 and **P<0.01 represent a significant difference between the values before and after drug injection.

Table 4. Effects of levromakalim on the peristaltic movement of the ureter with the kidney (n=5)

	Before injection		Vehicle (7% ethanol)		0.03 mg/kg, i.v. injection		10 min		60 min		0.3 mg/kg, i.v. injection		10 min	
	A	N	A	N	A	N	A	N	A	N	A	N	A	N
Mean	7.2	25.2	6.5	25.0	6.5	18.6*	6.4	22.8	6.4	24.8	0	0	6.5	10.2
S.E.	0.9	1.0	1.6	1.6	1.3	0.8	1.3	2.0	1.4	0.9	0	0	1.8	2.1

Tyrod's solution was injected into the ureter lumen through the cannula inserted into the ureter near the bladder. Time (min) indicates the time after injection of each dose of drug. The intraluminal pressure was recorded for about 60 min. The values except those for 10 min after injection of levromakalim (0.3 mg/kg, i.v.) were calculated mainly from the high-amplitude pressure signals, since it was difficult to count the low-amplitude pressure signals. The values 10 min after injection of levromakalim (0.3 mg/kg, i.v.) were calculated from the low-amplitude pressure signals. A: amplitude (cmH₂O) of the ureteral peristaltic pressure, N: number of pressure signals of ureteral peristaltic movement in 2 min. *P<0.05 represents a significant difference between the values before and after drug injection. As the values before injection of levromakalim (0.3 mg/kg, i.v.), the values 60 min after injection of levromakalim (0.03 mg/kg, i.v.) were used.

ureter, high-frequency and high- and low-amplitude pressure signals were recorded. After the ligation, high-amplitude pressure signals were recorded, and the pattern was very similar to that of ureteral pressure signals induced by infusion of fluid through the kidney-side cannula. Although connective tissue around the ureter near the kidney was carefully removed in order to pass the ligation thread under the ureter, the peristaltic pressure signal was quiescent in some experiments (B in Fig. 5). Levromakalim (0.03 mg/kg, i.v.) preferentially abolished the high-amplitude peristaltic pressure signals; and at a dose of 0.3 mg/kg, i.v., it reduced the basal pressure and abolished the low-amplitude pressure signals (Fig. 6). When the basal pressure returned to the level before injection of levromakalim, low-amplitude pressure signals resumed but the frequency was low, as compared with that before injection of the drug (Table 4).

Effect of levromakalim on urinary bladder contraction in anesthetized and conscious rats

In anesthetized rats after injection of levromakalim (0.3 mg/kg, i.v.), some bladder contractions appeared continuously in a short interval in five out of eight rats (Fig. 7). In the cystometrogram at 30 to 60 min after injection of levromakalim (0.3 mg/kg, i.v.), the peak pressure was reduced, but there was no change in the micturition interval as compared with those before injection. In conscious rats, levromakalim (0.3 mg/kg, i.v.) prolonged the micturition interval and increased the amount of solution excreted from the urethra during one micturition, but it did not reduce the peak pressure except at the first bladder contraction (Fig. 8). The effects of levromakalim on the bladder contraction in anesthetized and conscious rats are summarized in Table 5. Small

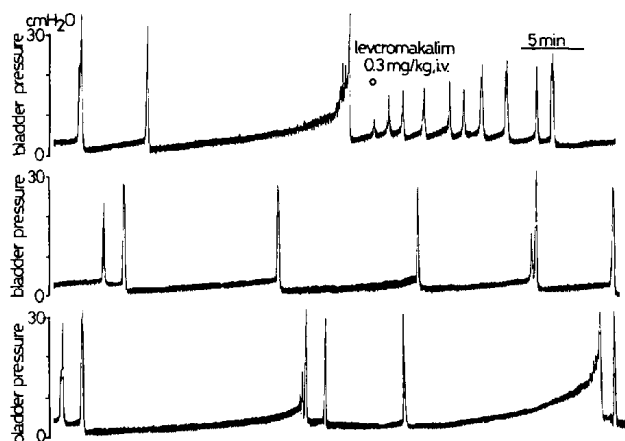


Fig. 7. Effect of levromakalim on the urinary bladder contraction induced by continuous infusion of fluid into the bladder in an anesthetized rat. Vertical bar: urinary bladder pressure (cmH₂O). Horizontal bar: 5 min. Lower trace is a continuous recording of the upper one.

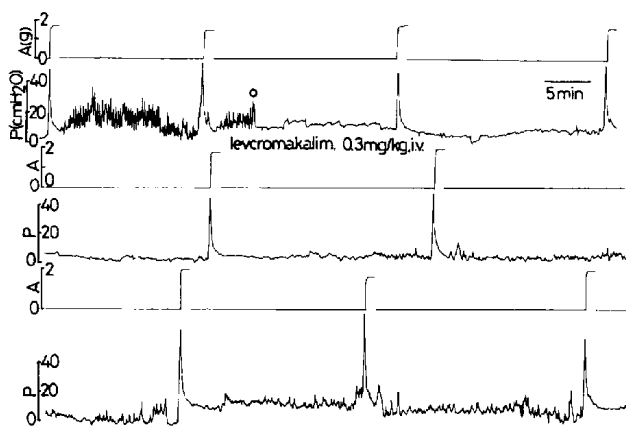


Fig. 8. Effect of levromakalim on the urinary bladder contraction induced by continuous infusion of fluid into the bladder in a conscious rat. Paired traces indicate the quantity of fluid excreted from the urethra (A: upper) and the cystometrogram (P: lower). Vertical bar: weight (A: g) and urinary bladder pressure (P: cmH₂O). Horizontal bar: 5 min. Lower paired traces are continuous recordings of the upper ones.

Table 5. Effect of levcromakalim on urinary bladder contraction in anesthetized and conscious rats

Anesthetized rats (n=8)																
	Levcromakalim (0.3 mg/kg, i.v.)															
	Before injection			30 min after injection			60 min after injection			120 min after injection						
	MI	TP	PP	MI	TP	PP	MI	TP	PP	MI	TP	PP				
Mean	10.4	4.8	26.0	7.2	2.8	22.0*	10.7	2.9	20.5*	13.7	2.8	23.7				
S.E.	1.7	1.6	5.5	10.0	0.9	5.0	0.9	0.5	4.9	1.7	0.8	5.0				
Conscious rats (n= 5)																
	Levcromakalim (0.3 mg/kg, i.v.)															
	Before injection				First micturition after injection				Second micturition after injection				120 min after injection			
	MI	TP	PP	A	MI	TP	PP	A	MI	TP	PP	A	MI	TP	PP	A
Mean	13.7	2.8	45.9	1.1	19.0*	5.3	31.2*	1.3**	17.2*	3.6	37.4	1.3*	18.8**	7.0	54.6	1.7*
S.E.	1.1	1.3	2.0	0.1	0.7	2.2	3.2	0.1	1.7	1.3	3.9	0.2	0.9	1.7	6.4	0.1

Bladder contraction was induced by continuous infusion of fluid into the bladder at a constant rate (approximately 0.8 ml/10 min). MI: micturition interval (min), TP: micturition threshold pressure (cmH₂O), PP: peak pressure during bladder contraction (cmH₂O), A: amount of fluid excreted by a single micturition (g). *P<0.05 and **P<0.01 represent a significant difference between the values before and after drug injection.

repetitive bladder contractions observed during the collecting phase in conscious rats disappeared after injection of levcromakalim (0.3 mg/kg, i.v.) for 122.4 ± 6.1 min (n=5).

Infusion of contrast medium into the bladder, instead of Tyrode's solution, induced bladder contractions similar to those induced by infusion of Tyrode's solution. When X-ray photographs were taken at 60 and 90 min after injection of levcromakalim (0.3 mg/kg, i.v.), contrast medium was not detected in the right ureter, which was kept intact.

DISCUSSION

In this experiment, we individually recorded the pressure signals induced by peristaltic movement of the ureter region (Figs. 2–4) and also the ureter with the kidney (Figs. 5 and 6) and studied the effect of levcromakalim on each movement. In our method, peristaltic movement of the ureter region distant from the pelvis had a high amplitude and a constant frequency (Figs. 2–4). On the other hand, as shown in Fig. 5, in the ureter along with the kidney, high and low amplitude pressure signals were recorded; and the result obtained after the ligation of the ureter near the kidney suggests that peristaltic movement of the ureter region near the pelvis had a low amplitude and a high frequency. As shown in Fig. 6, after injection of levcromakalim (0.3 mg/kg, i.v.), high-amplitude pressure signals had been abolished, but low-amplitude pressure signals were recorded when the basal pressure returned to the level before injection of levcromakalim. Low-amplitude pressure signals are not conducted to the pressure

transducer during reduction of the basal pressure in the ureter, and levcromakalim exerts a stronger inhibitory effect on peristaltic movement of the ureter region distant from the pelvis than that near the pelvis (Figs. 2, 4 and 6). It is considered that the inhibitory effect of levcromakalim on the ureteral pacemaker activity in the pelvis is weak or absent if low-amplitude pressure signals are generated by the activity. Levcromakalim relaxes the smooth muscle of the ureter near the pelvis, but not that distant from the pelvis, since the drug reduced basal pressure in the ureter alone with the kidney (Figs. 2, 4 and 6).

Although levcromakalim exerted an inhibitory effect on ureteral peristaltic movement, glibenclamide at a concentration allowing dissolution in 10% ethanol and i.p. injection of glibenclamide at a high dose (10 mg) and for more than 10 min did not antagonize the effect of levcromakalim (Figs. 2 and 4). If the site of action of these drugs is the ATP-dependent K⁺ channels described in many kinds of smooth muscle, the glibenclamide sensitivity of the K⁺ channel in ureter smooth muscle that generates peristaltic movement may be low. However, we can not neglect the possibility that the concentration of glibenclamide may have been too low for it to exert an effect on active sites of the ureter smooth muscles. Another type of K⁺ channel that is blocked by 4-aminopyridine or tetraethylammonium is reported (13). We did not investigate the effect of these drugs on ureteral peristaltic movement in the present experiment, since both drugs applied from the ureter lumen at high concentration did not affect it (10).

The effect of glibenclamide on vascular smooth muscle and a list of drugs that have effects on non-vascular smooth muscle have been reported by Zhang et al. (14),

but the effect of glibenclamide on the ureter smooth muscle contractility has not been reported. Glibenclamide did not affect ureteral peristaltic movement when it was applied intravascularly or intraperitoneally in the present experiment (Figs. 2 and 4) as well as from the ureter lumen in our previous report (10). We do not consider that the injection route chosen may have caused glibenclamide to exert no effect on ureteral peristaltic movement, since nifedipine inhibited ureteral peristaltic movement when it was intraperitoneally injected and applied from the ureter lumen (10).

The difference between the effect of the Ca antagonist nifedipine and that of the K⁺ channel opener levcromakalim was that nifedipine elevated the basal pressure and decreased the amplitude of pressure signals before their disappearance (Fig. 3). Nifedipine may relax the ureter smooth muscle, whereas levcromakalim may inhibit only the generation of contraction. Elevation of basal pressure after injection of nifedipine (3 mg/kg, i.p.) may be due to an increase in the amount of fluid flow caused by ureteral peristaltic movement into the ureter lumen whose muscle tonus was reduced by nifedipine. Nifedipine (10 mg/kg, i.p.) may abolish ureteral peristaltic movement before it reduces ureter muscle tonus, and therefore the elevation of basal pressure was low, as compared with that after injection of nifedipine (3 mg/kg, i.p.).

Levcromakalim even at a dose of 0.3 mg/kg, i.v. did not interrupt micturition (Figs. 7 and 8). The contractility of the detrusor muscle is more resistant to the inhibitory effect of levcromakalim than that of ureter circular smooth muscle. In anesthetized rats, small bladder contractions appeared continuously at short intervals after injection of levcromakalim (Fig. 7). Similar effects have also been observed upon injection of an antimuscarinic agent and a Ca antagonist (11, 12), and the generation of these bladder contractions is thought to be due to distention of the bladder wall. The reason why levcromakalim did not induce these small bladder contractions in conscious rats may be that one bladder contraction can expel almost all the fluid present in the bladder, and therefore the bladder wall is not distended by fluid thereafter. In conscious rats, levcromakalim suppressed the small bladder contractions occurring during the collecting phase (Fig. 8), as described by Malmgren et al. (6) in rats with bladder instability, and prolonged the micturition interval (Table 5). These results indicate that levcromakalim does not inhibit the bladder contraction but reduces the tonus of the detrusor muscle in rats and increases the bladder capacity. The amount of fluid excreted from the urethra was increased in conscious rats. In anesthetized rats, residual fluid in the bladder would have been increased, since the micturition interval did not change. In anesthetized rats, levcromakalim reduced the peak pressure

(Table 5). Urethral smooth muscle tonus would be partially reduced by anesthesia, since the peak pressure during micturition in anesthetized rats was lower than that in conscious rats (Table 5). This relaxation of urethral muscle tonus would appear when the inhibitory effects of anesthesia and levcromakalim were added.

The ureterovesical junction is able to close completely even after injection of levcromakalim (0.3 mg/kg, i.v.), since the vesicoureteral reflux of contrast medium is not observed.

In conclusion, levcromakalim preferentially inhibits the contractility of ureter smooth muscle that generates peristaltic movement, as compared with the detrusor smooth muscle and urethra smooth muscle. These effects of levcromakalim may be useful for the treatment of renal colic.

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