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The potency of KUL-7211, a selective ureteral relaxant, in isolated canine ureter: comparison with various spasmolytics

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Abstract We compared the potency of a selective ureteral relaxant KUL-7211 (β_2/β_3-adrenoceptor agonist; (-)-2-[4-(2-{[(1*S*,2*R*)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)phenyloxy]acetic acid) with those of various spasmolytics on contractions in isolated canine ureteral preparations. Drug effects were evaluated on the tonic contraction induced by KCl (80 mM) and on spontaneous, 1×10^{-5} M phenylephrine-, and 1×10^{-6} M $PGF_{2\alpha}$-induced rhythmic contractions in isolated canine ureteral preparations using a functional experimental technique. The potencies (pD_2 value) of the following drugs were compared: KUL-7211, tamsulosin (an $\alpha_{1A/1D}$-adrenoceptor antagonist), prazosin (an α_1-adrenoceptor antagonist), verapamil (a Ca^{2+}-channel blocker), butylscopolamine (a nonselective muscarinic antagonist), and papaverine (a phosphodiesterase inhibitor). The rank order of relaxing potencies against KCl-induced tonic contraction was KUL-7211 (6.60) > tamsulosin(5.90) > verapamil(5.70) > papaverine (4.88) > prazosin (4.54). The rank order of potencies for reductions in spontaneous rhythmic contractions was KUL-7211 (6.80) > verapamil(6.12) > papaverine(5.05). Conversely, high concentrations of the two α-adrenoceptor antagonists (tamsulosin and prazosin) and of butylscopolamine enhanced the spontaneous contractions, although at low concentrations (up to 1×10^{-6} M) they had no significant effects. For suppression of spasmogen-induced rhythmic contractions, the rank order of potencies was, against phenylephrine-induced contractions: KUL-7211 (6.95) > tamsulosin(6.26) > prazosin(5.68) > verapamil(5.64) > papaverine (5.03), and against $PGF_{2\alpha}$-induced contractions: KUL-7211 (7.05) > verapamil(6.70) > papaverine (5.27). Our results suggest that in dogs, the β_2/β_3-adrenoceptor agonist KUL-7211 is the most efficacious ureteral relaxant among the spasmolytics tested against various contractions. Possibly, KUL-7211 might be useful for promoting stone passage and relieving ureteral colic in urolithiasis patients.

Keywords KUL-7211 · Ureter · Relaxation · Stone passage

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Introduction

Urinary calculi are prime factors in ureteral obstruction, which produces an increase in intraluminal pressure within the upper urinary tract. This increase in intraureteral pressure leads to ureteral colic, sometimes with complications such as hydronephrosis or renal insufficiency and/or urinary tract infection [1]. It is therefore self-evident that promoting stone discharge helps stone patients avoid these problems.

In previous clinical studies, several spasmolytics have been shown to be effective for the promotion of stone passage and/or the relief of ureteral colic. For instance: (1) the use of a Ca^{2+} channel-blocking agent increases stone expulsion rate and decreases stone expulsion time [2, 3], (2) the use of papaverine may relieve ureteral colic [4], and (3) treatment with tamsulosin, an $\alpha_{1A/1D}$-adrenoceptor antagonist, is effective at facilitating stone passage in patients with a lower ureteral stone [2, 5]. Thus, it has become evident that spasmolytics can be useful for facilitating the passage of ureteral stones with the subsequent relief of ureteral colic.

β-adrenoceptors exist in the ureters of humans, rabbits, dogs, and pigs, where they play a role in the relaxation of the ureter [6–8]. We have already demonstrated that β-adrenoceptor agonists significantly decrease the elevated intraureteral pressure and eliminate urine stagnation above the obstruction in an acute ureteral-obstruction model in anesthetized dogs [9]. Later, Miyatake et al. [10] demonstrated in anesthetized

rabbits that relaxation of ureteral smooth muscle by β-adrenergic stimulation reduced ureteral wall tension. This diminished the friction between an artificial ureteral stone and the ureter. Interestingly, Weiss [11] showed that stone passage is promoted by reducing friction as well as by enhancing ureteral peristalsis and diuresis. These findings suggest that a really effective β-adrenoceptor agonist could serve as a new drug for urolithiasis patients, to promote stone passage and relieve ureteral colic.

Recently, we developed the selective ureteral relaxant KUL-7211 (a β_2/β_3-adrenoceptor agonist) [12]. The aim of the present study was to compare KUL-7211 with various spasmolytics (the α_1-adrenoceptor antagonists tamsulosin and prazosin, the Ca^{2+}-channel blocker verapamil, the nonselective muscarinic antagonist butylscopolamine, and the phosphodiesterase inhibitor papaverine) in terms of their ureteral-relaxing potency on various contractions in isolated canine ureteral preparations.

Materials and methods

Animals

This study was conducted according to guidelines approved by the Laboratory Animal Committee of Kissei Pharmaceutical Company Ltd., and it conformed to current Japanese law. Male Beagle dogs (Nihon Nosan Kogyo, Yokohama, Japan) weighing 9.8–15.3 kg were housed individually. They were maintained under a 12-h light-dark cycle with free access to water and standard laboratory food until the day of the experiment.

Tissue preparation and experimental protocol

Dogs were anesthetized with sodium pentobarbital (30 mg/kg i.v.), then sacrificed by rapid exsanguination. Tissue preparation and the experimental protocol were described in detail in our previous publication [12]. Each preparation was exposed to only one test drug.

KCl-induced tonic contractions in isolated tubular preparations of the ureter

The left and right ureters were isolated, and after removal of fat and blood vessels, each ureter was cut into tubular segments approximately 20 mm long. For a given experiment, one of these segments was suspended in a 10 ml organ bath containing Krebs solution (composition: 118.1 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4 \cdot 7H_2O$, 25.0 mM $NaHCO_3$, 1.2 mM KH_2PO_4, and 11.1 mM glucose). This was maintained at 37°C and continuously gassed with a mixture of 95% O_2 and 5% CO_2. Changes in tissue tension were recorded isometrically using a force-displacement transducer and measuring system (TB-611T, AP-601G and RPM-6004; Nihon Kohden) connected to a thermowriting rectigraph (Recti-Horiz-8K; GE Marquette Medical Systems). Ureteral preparations were allowed to equilibrate for 1 h after the establishment of an initial resting tension of 2–3 mN. The actions of drugs were initially evaluated by testing their effects on the 80 mM KCl-induced tonic tension, which was a maximum contraction in some preparations and submaximum in others [7]. The high-potassium bathing solution (80 mM KCl) was prepared by adding 0.2 ml of a 4 M KCl stock solution to the organ bath. After the KCl-induced tonic contraction had stabilized, a given drug was added in 0.5-log increments every 2.5 min. Each drug-induced ureteral relaxation is expressed here as a percentage of the maximum response to 1×10^{-5} M forskolin, an adenylate cyclase activator, which was added at the end of the experiment. The KCl-induced contraction of the ureter showed a slight, progressive decrease during the course of the experiment when, instead of drug solution, the same volume of distilled water (vehicle) was added to the organ bath every 2.5 min. Therefore, data were corrected by reference to the vehicle-treated control level at each time-point. The intrinsic activity (I.A.) value for a given drug was calculated as the ratio between the maximum ureteral relaxation induced by that drug and that elicited by 1×10^{-5} M forskolin.

Spontaneous or spasmogen-induced rhythmic contractions in isolated spiral preparations of the ureter

The left and right ureters were isolated, and after removal of fat and blood vessels, each ureter was cut into spiral segments (5×20 mm each). An initial resting tension of 2–3 mN was placed on the ureteral segment, and it was then allowed to equilibrate for 120 min. After the spontaneous rhythmic contractions had stabilized, a given drug was added in 1.0-log increments every 5 min. Ureteral preparations that showed no spontaneous rhythmic contractions within 120 min were subjected to spasmogen treatment (1×10^{-5} M phenylephrine or 1×10^{-6} M $PGF_{2\alpha}$). In these preparations, the effects of drugs were evaluated on the 1×10^{-5} M phenylephrine or 1×10^{-6} M $PGF_{2\alpha}$-induced contractions, which were found to be either maximum or submaximum contractions in our preliminary experiment (data not shown). To this end, after the spasmogen-induced rhythmic contractions had stabilized, a given drug was added in 1.0-log increments every 5 min. The effect of a given drug on ureteral motility was evaluated by calculating the percentage change in total amplitude of all the contractions occurring during a 5-min period. Maximum inhibition of the rhythmic contractions was adopted as the intrinsic activity.

Drugs

KUL-7211 and tamsulosin were synthesized in our laboratory (Kissei Pharmaceutical, Hotaka, Japan).

Prazosin, butylscopolamine, verapamil, and papaverine were obtained from Sigma (St. Louis, Mo.), phenylephrine and forskolin from Wako (Osaka, Japan), and sodium pentobarbital and $PGF_{2\alpha}$ (Prostarmon) from Dainippon Pharmaceutical (Osaka, Japan) and Ono Pharmaceutical (Osaka, Japan), respectively. KUL-7211 was dissolved in distilled water together with an equivalent molarity of HCl. Forskolin and prazosin were dissolved in 100% dimethyl sulfoxide (DMSO; Nacalai tesque, Kyoto, Japan), the other agents in distilled water.

Data analysis

The pD_2 value was estimated by taking the negative logarithm of the molar concentration required to produce 50% of the maximum relaxation (or inhibition) elicited by each drug (EC_{50} value). All results are expressed as the mean ± standard error of the mean (SEM).

Results

Effects of drugs on KCl-induced tonic contraction

Once canine ureteral tubular preparations had achieved stable tonic contractions in the presence of 80 mM KCl, a marked relaxation of this contraction was induced by KUL-7211 in a concentration-dependent manner (Fig. 1, Table 1). In terms of the pD_2 value, verapamil was less potent than KUL-7211. Although the two α-adrenoceptor antagonists (tamsulosin and prazosin) and papaverine also relaxed the ureter, they had only weak effects except at higher concentrations. In contrast, butylscopolamine had no effect at all on the KCl-induced contraction.

Effects of drugs on spontaneous rhythmic contractions

KUL-7211, verapamil, and papaverine concentration-dependently reduced spontaneous rhythmic contractions in the canine ureter (Fig. 2, Table 1), the rank order of potency (pD_2 values) being KUL-7211 > verapamil > papaverine. In sharp contrast, high concentrations of the two α-adrenoceptor antagonists (tamsulosin and prazosin) and of butylscopolamine enhanced the ureteral contractions.

Effects of drugs on phenylephrine-induced rhythmic contractions

KUL-7211 markedly suppressed the ureteral contractions induced by 1×10^{-5} phenylephrine (Fig. 3, Table 1). Although tamsulosin, prazosin, and verapamil each produced a concentration-dependent reduction, their potencies were less than that of KUL-7211. Papaverine had only a slight inhibitory effect. The higher

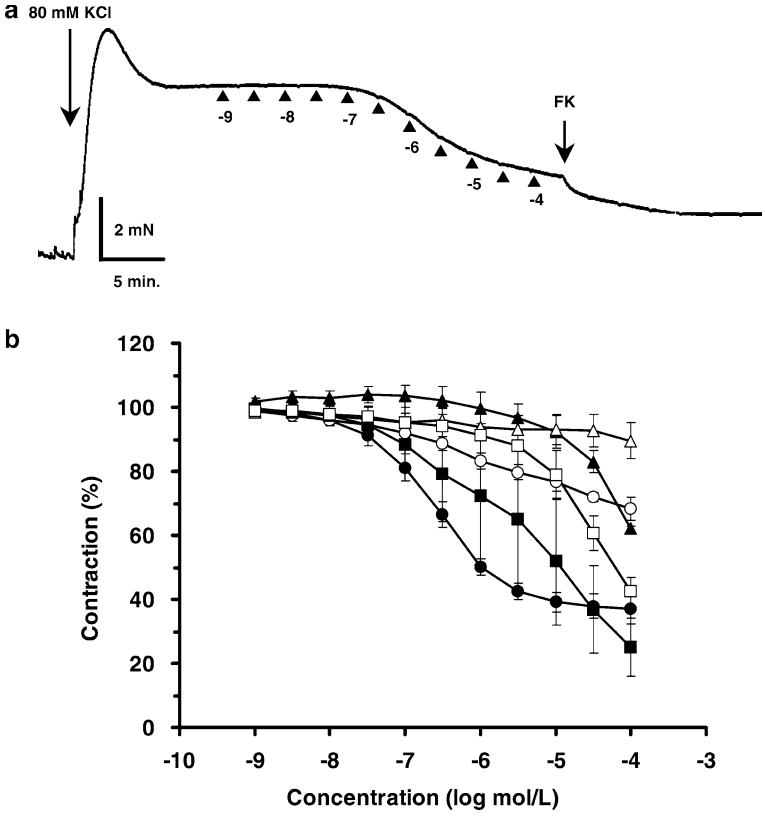

Fig. 1 Effects of KUL-7211 and other spasmolytics on the contraction induced by 80 mM KCl in isolated canine ureteral tubular preparations. **a** Representative tracing of effect of KUL-7211 on the contraction induced by 80 mM KCl, with KUL-7211 concentrations shown as the logarithm of the molar concentration. **b** Concentration-response curves for effects of KUL-7211 (*filled circles*), tamsulosin (*open circles*), prazosin (*filled triangles*), butylscopolamine (*open triangles*), verapamil (*filled squares*), and papaverine (*open squares*) on the contraction induced by 80 mM KCl. Each value is expressed as a percentage of the response to 1×10^{-5} M forskolin (*FK*). Means ± SEM from four experiments

Table 1 pD_2values and intrinsic activity (I.A.) obtained for each drug against various contractions in canine ureteral preparations. Data from 4–6 separate experiments

Drug	KCl		Spontaneous		Phenylephrine		$PGF_{2\alpha}$	
	pD_2	I.A.	pD_2	I.A.	pD_2	I.A.	pD_2	I.A.
KUL-7211	6.60 ± 0.19	0.64 ± 0.04	6.80 ± 0.23	0.95 ± 0.04	6.95 ± 0.33	1.00 ± 0.00	7.05 ± 0.32	0.95 ± 0.04
Tamsulosin	5.90 ± 0.34	0.33 ± 0.02	-	0.16 ± 0.08	6.26 ± 0.23	0.89 ± 0.05	-	0.15 ± 0.06
Prazosin	4.54 ± 0.12	0.38 ± 0.01	-	0.07 ± 0.03	5.68 ± 0.16	0.85 ± 0.07	-	0.18 ± 0.07
Butylscopolamine	-	0.12 ± 0.04	-	0.17 ± 0.06	-	0.19 ± 0.09	-	0.09 ± 0.04
Verapamil	5.70 ± 0.46	0.75 ± 0.09	6.12 ± 0.27	1.00 ± 0.00	5.64 ± 0.41	0.96 ± 0.04	6.70 ± 0.32	1.00 ± 0.00
Papaverine	4.88 ± 0.18	0.58 ± 0.05	5.05 ± 0.21	0.90 ± 0.08	5.03 ± 0.03	0.68 ± 0.16	5.27 ± 0.32	0.93 ± 0.04

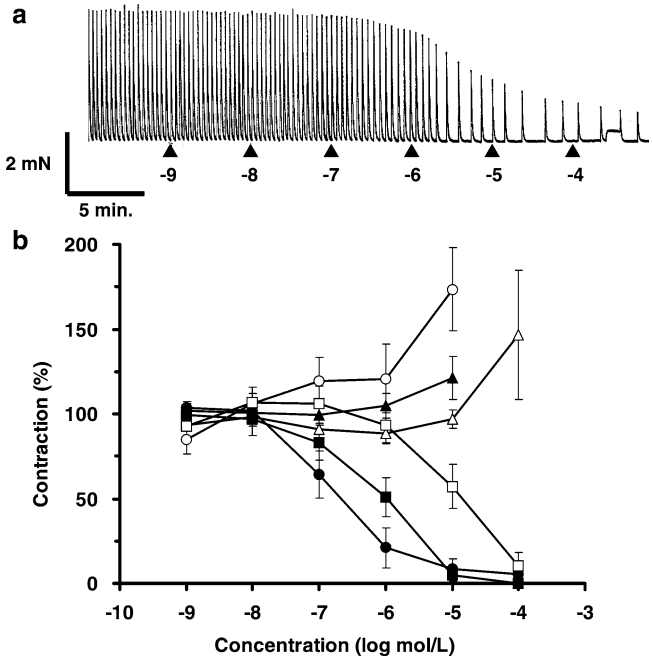

Fig. 2 Effects of KUL-7211 and other spasmolytics on spontaneous rhythmic contractions in isolated canine ureteral spiral preparations. **a** Representative tracing of the effect of KUL-7211 on spontaneous rhythmic contractions, with KUL-7211 concentrations shown as the logarithm of the molar concentration. **b** Concentration-response curves for effects of KUL-7211 (*filled circles*), tamsulosin (*open circles*), prazosin (*filled triangles*), butylscopolamine (*open triangles*), verapamil (*filled squares*), and papaverine (*open squares*) on spontaneous rhythmic contractions. Each value is expressed as the percentage change in the sum of the amplitudes of all contractions occurring during a 5-min period. Means ± SEM from four to six experiments

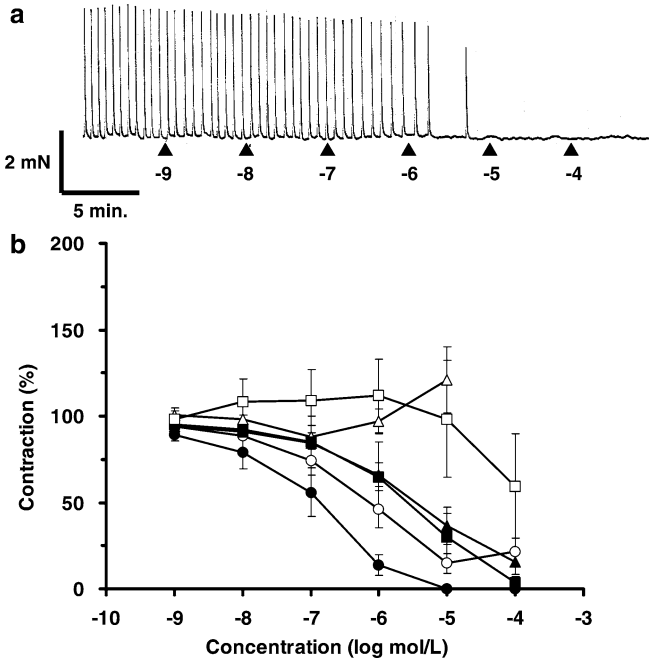

Fig. 3 Effects of KUL-7211 and other spasmolytics on 1×10^{-5} M phenylephrine-induced rhythmic contractions in isolated canine ureteral spiral preparations. **a** Representative tracing of effect of KUL-7211 on phenylephrine-induced rhythmic contractions, with KUL-7211 concentrations shown as the logarithm of the molar concentration. **b** Concentration-response curves for the effects of KUL-7211 (*filled circles*), tamsulosin (*open circles*), prazosin (*filled triangles*), butylscopolamine (*open triangles*), verapamil (*filled squares*), and papaverine (*open squares*) on phenylephrine-induced rhythmic contractions. Each value is expressed as the percentage change in the sum of the amplitudes of all contractions occurring during a 5-min period. Means ± SEM from four or five experiments

concentrations of butylscopolamine ($>1\times10^{-5}$ M) enhanced the phenylephrine-induced contraction.

Effects of drugs on $PGF_{2\alpha}$-induced rhythmic contractions

The $PGF_{2\alpha}$-induced contractions of the canine ureter were markedly attenuated by KUL-7211 and verapamil, and also by papaverine (Fig. 4, Table 1). The rank order of potencies was KUL-7211 > verapamil > papaverine. Prazosin had no effect at all, but tamsulosin and butylscopolamine enhanced the contractions at higher concentrations.

Discussion

For some time, β-adrenoceptors have been known to exist in the mammalian ureter and to play an important role in its relaxation [11]. However, we previously reported species variability in the β-adrenoceptor

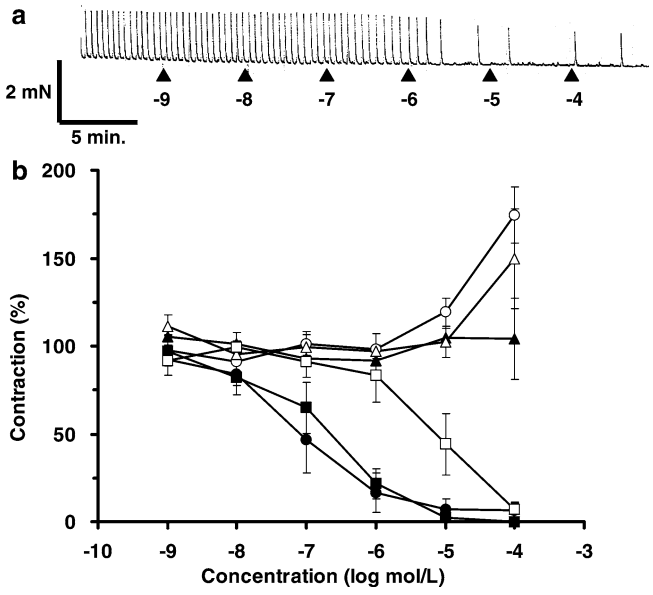

Fig. 4 Effects of KUL-7211 and other spasmolytics on 1×10^{-6} M $PGF_{2\alpha}$-induced rhythmic contractions in isolated canine ureteral spiral preparations. **a** Representative tracing of effect of KUL-7211 on $PGF_{2\alpha}$-induced rhythmic contractions, with KUL-7211 concentrations shown as the logarithm of the molar concentration. **b** Concentration-response curves for effects of KUL-7211 (*filled circles*), tamsulosin (*open circles*), prazosin (*filled triangles*), butylscopolamine (*open triangles*), verapamil (*filled squares*), and papaverine (*open squares*) on $PGF_{2\alpha}$-induced rhythmic contractions. Each value is expressed as the percentage change in the sum of the amplitudes of all contractions occurring during a 5-min period. Means ± SEM from four or five experiments

subtypes mediating relaxation of the ureter (mainly the β_1-adrenoceptor in rats, only the β_2-adrenoceptor in rabbits, mainly the β_3-adrenoceptor in dogs, and both β_2- and β_3-adrenoceptors in pigs) [7, 8]. Since in humans ureteral relaxation is mediated by both β_2- and β_3-adrenoceptors [6], KUL-7211, a new β_2/β_3-adrenoceptor agonist recently developed as a ureteral relaxant in our laboratory, would be expected to be much more suitable for inducing human ureteral relaxation than other β-adrenoceptor agonists [12].

In the present study, KUL-7211, the Ca^{2+}-channel blocker verapamil, and the phosphodiesterase inhibitor papaverine reduced both spontaneous and spasmogen-induced ureteral contractions, although in each case the potency of KUL-7211 was greater than those of verapamil and papaverine. The two α_1-adrenoceptor antagonists tested, tamsulosin and prazosin, induced concentration-dependent reductions only on the phenylephrine-induced ureteral contraction, and had no effects at all on the other contractions examined in this study. Neither ureteral relaxation nor suppression was observed with butylscopolamine. We therefore demonstrated that for inducing ureteral relaxation, KUL-7211 has the greatest potency among the six drugs tested.

It is well known that human ureteral contractions can be elicited by various neurotransmitters or other substances, such as norepinephrine, $PGF_{2\alpha}$, 5-hydroxytryptamine, histamine, and KCl [13–17]. However, less is known about the major neurotransmitters responsible for human ureteral contraction in vivo. In our preliminary experiments on dogs, among the spasmogens tested (KCl, phenylephrine, histamine, carbachol, 5-hydroxytryptamine, and $PGF_{2\alpha}$) only KCl, phenylephrine, and $PGF_{2\alpha}$ proved able to induce tonic or rhythmic contractions that were stable enough for us to use to evaluate drug efficacy (data not shown). Since KUL-7211 exhibited stronger ureteral relaxing effects on spontaneous and spasmogen-induced contractions than the other five spasmolytics tested in this study, it seems highly likely that KUL-7211 would produce significant relaxation on the ureteral contractions induced by various neurotransmitters and other substances in the human ureter.

In recent years, several studies have focused on ureteral relaxants because these drugs can be expected to facilitate stone passage in urolithiasis patients. For instance, the β-adrenoceptor agonist isoproterenol, which is a powerful ureteral relaxant [7], was found to reduce the friction between an artificial ureteral stone and the ureteral smooth muscle in anesthetized rabbits [10]. Since friction between a stone and the ureteral wall greatly hinders the passage of the stone [11], reducing such friction by the use of a relaxant would promote stone passage. Recently, tamsulosin and the Ca^{2+}-channel blocker nifedipine have been reported to facilitate stone passage in patients with lower ureteral stones [2, 5]. In the present study, tamsulosin (α_1-adrenoceptor antagonist) and verapamil (Ca^{2+}-channel blocker) reduced the contractions induced by phenylephrine and by various spasmogens, respectively, in isolated canine ureters. The mechanisms underlying the facilitation of stone passage by tamsulosin and nifedipine in the clinical setting seem likely to be related to their relaxing effects on the ureter. In the present canine experiments, we demonstrated that KUL-7211 was more potent at inducing ureteral relaxation than the other smooth muscle relaxants, such as tamsulosin and verapamil. We therefore suggest that KUL-7211 might be very useful for promoting stone passage in urolithiasis patients, and indeed that it might be more effective than the other ureteral relaxants tested.

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