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Regulation of Ba^{2+}-Induced Contraction of Murine Ureteral Smooth Muscle

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This study was designed to characterize ureteral smooth muscle motility and also to study the effect of forskolin (FSK) and isoproterenol (ISO) on smooth muscle contractility in murine ureter. High K^+ (50 mM) produced tonic contraction by 0.17±0.06 mN (n=19). Neuropeptide and neurotransmitters such as serotonin (5 μM), histamine (20 μM), and carbarchol (CCh, 10 ~ 50 μM) did not produce significant contraction. However, CCh (50 μM) produced slow phasic contraction in the presence of 25 mM K^+. Cyclopiazonic acid (CPA, 10 μM), SR Ca^{2+}-ATPase blocker, produced tonic contraction (0.07 mN). Meanwhile, inhibition of mitochondria by protonophore carbnylcyanide m-chlorophenylhydrazone (CCCP) also produced weak tonic contraction (0.01 mN). The possible involvement of K^+ channels was also pursued. Tetraethyl ammonium chloride (TEA, 10 mM), glibenclamide (10 μM) and quinidine (20 μM) which are known to block Ca^{2+}-activated K^+ channels (K_{Ca} channel), ATP-sensitive K^+ channels (K_{ATP}) and nonselective K^+ channel, respectively, did not elicit any significant effect. However, Ba^{2+} (1 ~ 2 mM), blocker of inward rectifier K^+ channels (K_{IR} channel), produced phasic contraction in a reversible manner, which was blocked by 1 μM nicardipine, a blocker of dehydropyridine-sensitive voltage-dependent L-type Ca^{2+} channels ($VDCC_L$) in smooth muscle membrane. This Ba^{2+}-induced phasic contraction was significantly enhanced by 10 μM cyclopiazonic acid (CPA) in the frequency and amplitude. Finally, regulation of Ba^{2+}-induced contraction was studied by FSK and ISO which are known as adenylyl cyclase activator and β-adrenergic receptor agonist, respectively. These drugs significantly suppressed the frequency and amplitude of Ba^{2+}-induced contraction ($p < 0.05$). These results suggest that Ba^{2+} produces phasic contraction in murine ureteral smooth muscle which can be regulated by FSK and β-adrenergic stimulation.

Key Words: Ureter, Murine smooth muscle, Ba^{2+}, Isoproterenol (ISO), Cyclopiazonic acid (CPA)

INTRODUCTION

Urinary storage and micturition require a coordinated series of motility in the various regions of the urinary systems including ureter. In the phase of urine storage, urethral pressure exceeds the intravesical pressure in order to maintain continence, and low pressures within the bladder facilitate transport of urine from the kidney to the lower urinary duct. Such harmonious coordination of smooth muscle contractility in every urinary system permits urine flow from the kidney to urethra through bladder.

In most mammals except human or pig, isolated ureter is usually quiescent and the removal of renal pelvis decreases frequency of spontaneous motility in distal area of ureter (Cole et al, 1988; Constantinou et al, 1978). Since spontaneous contraction propagates distally along the ureter, motility of upper ureter has been known to be myogenic, and its c-*Kit* activity, which can be a criterion for detection of pacemaker cell and is essential for peristalsis, has recently been identified as similar to gastrointestinal (GI) tract (David et al, 2005; Huizinga et al, 1995). In GI tract, interstitial cells of Cajal (ICC) which lie near the border of the myenteric, submucous plexuses and in circular muscle layer are responsible for the initiation and propagation of myogenic slow waves throughout their organs (Huizinga et al, 1995). Likewise, migrating peristaltic contractility in mammalian upper ureter is now also regarded as a source for propelling urine from kidney to bladder, where it is stored until micturition.

Contractility of smooth muscles is regulated by several factors. Activation of muscarinic cholinergic receptor and β-adrenergic receptors as well as production of nitric oxide (NO) are well known mechanisms to regulate ureteral contraction (Danuser et al, 2001; Iselin et al, 1996; Miyatake et al, 2001; Murakami et al, 2000; Tomiyama et al, 1998; Wanajo et al, 2004; Wheeler et al, 1995). Furthermore, β-adrenoceptors have been reported to exist in

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the mammalian ureter and their stimulation produces ureteral relaxation (Miyatake et al, 2001; Murakami et al, 2000; Tomiyama et al, 1998; Wanajo et al, 2004). At cellular level, it is also known that stimulation of β-adrenoceptors activates adenylate cyclase, increases cyclic AMP (cAMP), and then produces smooth muscle relaxation (Andersson, 2004; Bylund et al, 1994; Yamaguchi et al, 2002). Therefore, regulation of ureteral smooth muscle contractility by cytosolic factors such as cAMP seems to be important mechanism to elucidate. Clinically, ureteral relaxing agent will be a useful tool facilitating spontaneous discharge of stones and relief of colicky pain.

In general, smooth muscle contractility is known to be accompanied by increase of intracellular Ca^{2+} ($[Ca^{2+}]_i$). Being released from the sarcoplasmic reticulum (SR) (Burdyga et al, 1995; Imaizumi et al, 1989), Ca^{2+} enters through voltage-dependent L-type Ca^{2+} channels ($VDCC_L$) on smooth muscle membrane (Imaizumi et al, 1989; Lang, 1990). Therefore, both pathways for Ca^{2+} entry are essential for force generation in smooth muscles. Regulation of excitability in smooth muscle via changes of membrane potential by K^+ channels such as Ca^{2+}-activated K^+ channels (K_{Ca} channel), ATP-sensitive K^+ channels (K_{ATP}) and A-type K^+ channels is also important since activation of these channels counteracts membrane excitability (Burdyga & Wray, 2005; Hernandez et al, 1997; Knot et al, 1996; Latorre et al, 1989; McCarron & Halpern, 1990; Nelson, 1993; Smith et al, 2002). As well as these K^+ conductances, inwardly rectifier K^+ channels (K_{IR} channel) which is known to underlie vasodilation and modulation of basal tone in artery were also reported (Knot et al, 1996; McCarron & Halpern, 1990). In mammalian ureter, several ionic conductances including K^+ channels have also been reported (Hernandez et al, 1997; Lang, 1990; Smith et al, 2002). In rat and guinea-pig ureter, three types of K^+ channels, such as K_{Ca} channel, K_{ATP} channels and A-type K^+ channels, have been identified (Smith et al, 2002; Sui & Kao, 1997), and the enhancing effect of tetraethylammonium (TEA), which is known to block K_{Ca} channel, on human ureteral contraction has also been reported (Soares de Moura & de-Lemos Net, 1996). However, the role of these channels in murine ureteral motility has not yet been studied.

Because of these reasons, this study was designed first to characterize ureteral smooth muscle motility and then to evaluate which kind of K^+ channel is important in its modulation. Finally, we studied the effect of forskolin (FSK) and isoproterenol (ISO) on smooth muscle contractility of murine ureter.

METHODS

Measurement of mechanical activity

Mulvany myograph was used for the recording of isometric tension from murine ureter (Oostendorp et al, 2000). All experiments were performed in accordance with the guidelines for the animal care and use approved by the Chungbuk National University. Either gender of ICR mice (30 g) was anesthetized with ether and exsanguinated by cervical dislocation. Ureters, including kidneys in both sides, were excised and placed in phosphate-buffered Tyrode's solution. Then, surrounding connective tissues in those ureters were gently removed under a stereomicroscope. Then, ureteral ring was prepared (1.5 mm in width) from one third upper region of ureter. Each ring was mounted horizontally in organ bath containing physiological salt solution (PSS) (*See Solutions and Drugs*). Each ring was stretched passively to a resting tension (0.5 mN for 2 and half hours). After further equilibration for 90~120 minutes, contractile response of the strip to the 50 mM K^+ containing solution was repeated two or three times until the responses were reproducible.

Solutions and Drugs

Phosphate-buffered Tyrode's solution contained (mM): NaCl 145, KCl 5, $MgCl_2$ 2, $CaCl_2$ 2, glucose 10, NaH_2PO_4 0.42, Na_2HPO_4 1.81, HEPES 10, pH 7.4. CO_2/bicarbonate-buffered Tyrode solution contained (in mM): NaCl 122, KCl 4.7, $MgCl_2$ 1, $CaCl_2$ 2, $NaHCO_3$ 15, KH_2PO_4 0.93, and glucose 11 (pH 7.3~7.4, bubbled with 5 % CO_2/95 % O_2). Equimolar concentration of Na^+ was replaced by K^+ for making high K^+ (25 or 50 mM) solution. All drugs used in this study were purchased from Sigma.

Statistics

Data are expressed as means±standard errors of the mean (means±SEM). The Student's t-test was used to evaluate differences in data wherever appropriate. p value less than 0.05 was taken as statistically significant.

RESULTS

Characterization of murine ureteral smooth muscle contractility

Changes of contraction in murine ureteral circular muscle were measured by Mulvany myograph (*See Method*). Isolated ureteral ring was mounted in isometric force transducer, as shown in Fig. 1A. Isolated ureteral smooth muscle did not show any spontaneous contraction, except two cases tested. As shown in Fig. 1B, ureteral smooth muscle showed spontaneous phasic contraction, and its amplitude and frequency were 0.08 mN and 0.45 cycles/min, respectively (n=2). To study the effect of neuropeptide and neurotransmitters on murine mureteral motility, histamine (20 μM), carbachol (CCh, 10~50 μM) and serotonin (5 μM) were applied, however, no significant effect was observed. On the other hand, CCh (20 μM) changed the contraction patterns induced by high K^+. Even though data are not shown, application of 50 mM high K^+ solution produced tonic contraction by 0.19±0.06 mN (n=8, data not shown). However, tonic contraction induced by high K^+ solution was changed to slow phasic contraction by application of CCh (20 μM) in the presence of 25 mM high K^+ solution (Fig. 1C). Its amplitude, frequency and duration were 0.17±0.09 mN, 0.09±0.01 cycles/min and 10±0.8 min, respectively (n=5). As shown in Fig. 1D-F, we also studied the effect of CPA and CCCP on murine ureter. CPA (10 μM) and CCCP (5 μM) produced tonic contraction and its amplitudes were 0.07±0.03 mN and 0.01±0.003 mN, respectively (n=5, respectively).

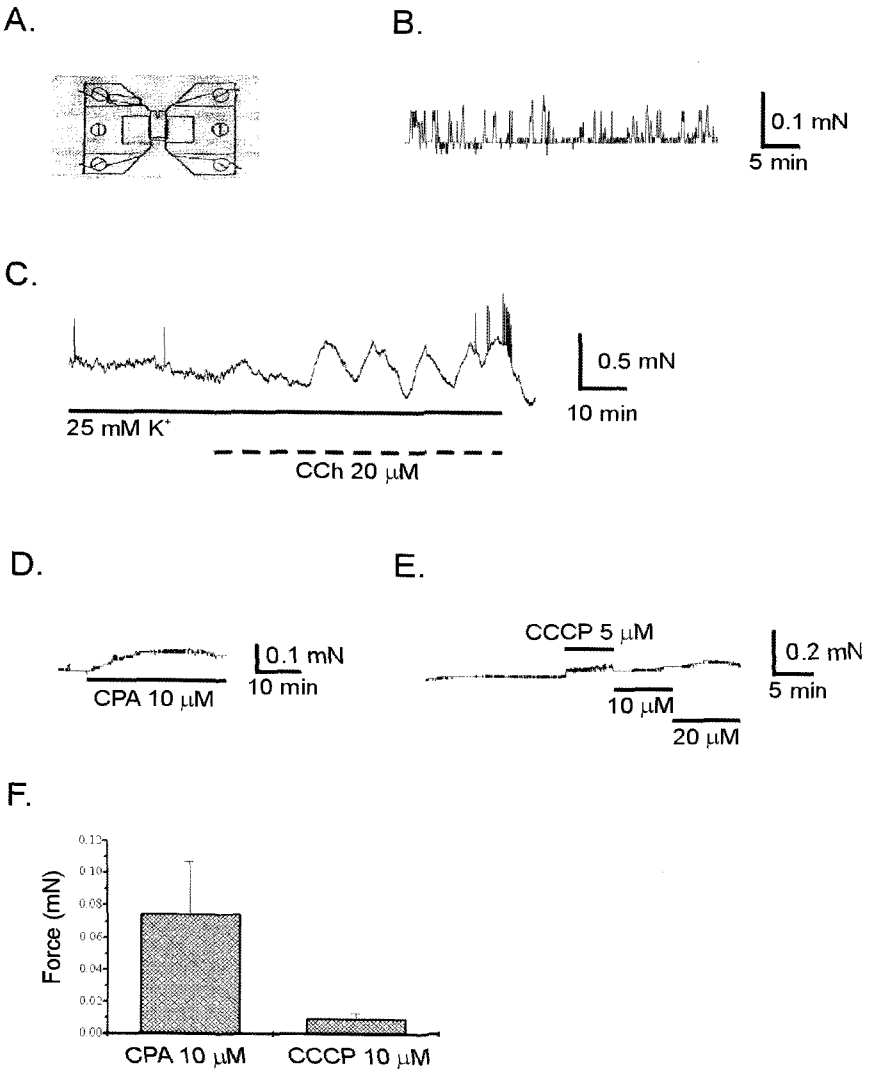

Fig. 1. Characterization of murine ureteral smooth muscle contractility. (A) Diagram for measuring isometric contraction of murine ureteral contraction using Mulvany myograph. (B) In two cases, spontaneous contraction was recorded in murine ureteral smooth muscle. (C) CCh-induced slow phasic contraction was observed in the presence of 25 mM high K^+ solution. In (D) and (E), CPA (10 μM) and CCCP (5 μM) increased basal tone of murine ureteral smooth muscle by 0.07 and 0.01 mN, respectively (n=5). (F) Bar graph shows summary of (D) and (E).

Ba^{2+}-induced contraction in murine ureteral smooth muscle

Effects of several potassium channel blockers, such as tetraethylammonium chloride (TEA), glibenclamide and quinidine which are known to block K_{Ca} channel, K_{ATP} channels and nonselective K^+ channels on murine ureteral smooth muscle contractility, were studied. However, TEA (10 mM), glibenclamide (10 μM) and quinidine (20 μM) did not show any contractile effect (n=7, 4 and 4, respectively), whereas Ba^{2+} which is known to block K_{IR} channel produced phasic contraction in a reversible manner. As shown in Fig. 2A, Ba^{2+} (2~4 mM) produced phasic contraction and the amplitude of contraction induced by 2 and 3 mM Ba^{2+} were 0.5±0.11 mN (n=21) and 0.6±0.11 mN (n=3, respectively, and its frequencies were 2.6±0.3 cycles/min (n=21) and 2.0±0.76 cycles/min (n=3), respectively. Fig. 2C shows that Ba^{2+}-induced phasic contraction in smooth muscle membrane was completely inhibited by nicardipine (1 μM) which is known to block dihydrohpyridine-sensitive voltage-dependent L-type Ca^{2+} channels ($VDCC_L$).

Effect of cyclopiazonic acid (CPA) on Ba^{2+}-induced contraction in murine ureteral smooth muscle

Ba^{2+}-induced contraction in murine ureteral smooth muscle was significantly enhanced by CPA which is known to block SR Ca^{2+}-ATPase (Fig. 3A). The amplitude and frequency of 2 mM Ba^{2+}-induced phasic contraction were

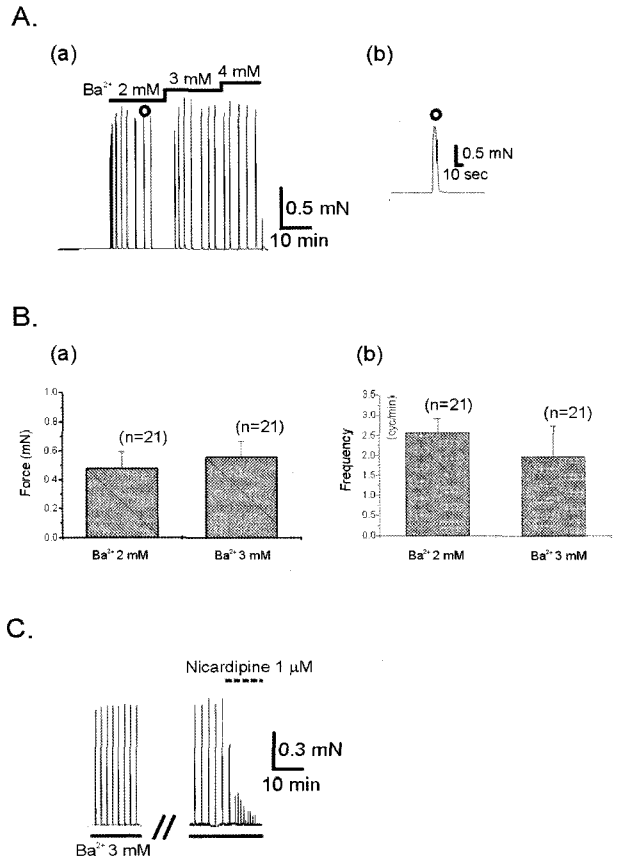

Fig. 2. Ba^{2+}-induced contraction in murine ureteral smooth muscle. (A) Application of Ba^{2+} produced phasic contraction in murine ureteral smooth muscle. Inset in (Ab) shows the expanded trace of Ba^{2+}-induced phasic contraction from Aa. The amplitude and frequency of Ba^{2+}-induced contraction by 2 and 3 mM Ba^{2+} are summarized in (B). (C) Blocking effect of nicardipine (1 μM) on Ba^{2+}-induced contraction in murine ureteral smooth muscle is shown. nicardipine (1 μM) completely inhibited Ba^{2+}-induced phasic contraction.

0.35±0.08 mN and 2.7±0.74 cycles/min, respectively (n=21). These contractions were significantly increased, corresponding to 212±59.8% and 303±23.7% of the control (n=21). Ba^{2+}-induced phasic contraction and the enhanced Ba^{2+}-induced contractility by CPA were completely blocked by 1 μM nicardipine (Fig. 3C).

Effect of forskolin (FSK) and isoproterenol (ISO) on Ba^{2+}-induced contraction in murine ureteral smooth muscle

Regulation of Ba^{2+}-induced contraction by FSK and ISO, which is known as adenylyl cyclase activator and β-adrenergic receptor agonist, was studied. Both FSK and ISO significantly suppressed the frequency and amplitude of Ba^{2+}-induced contraction ($p<0.05$). FSK (10 μM) decreased the amplitude and frequency of Ba^{2+}-induced contraction to 27±9.6% and 11±10.7% of the control (n=5), while ISO (4 μM) suppressed the amplitude and frequency of Ba^{2+}-induced contraction to 64±15.7% and 67±14.4% of the control (n=5).

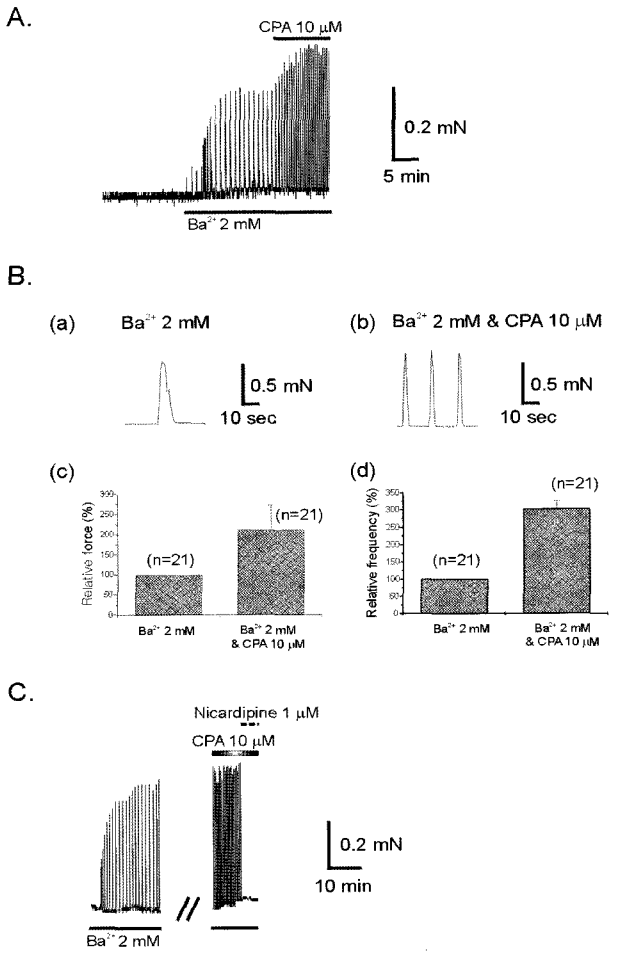

Fig. 3. Effect of cyclopiazonic acid (CPA) on Ba^{2+}-induced contraction in murine ureteral smooth muscle. (A) Ba^{2+}-induced contraction in murine ureteral smooth muscle was significantly enhanced by CPA. CPA (10 μM) increased the amplitude and frequency of Ba^{2+}-induced phasic contraction in a reversible manner. Representative traces of the effects of Ba^{2+} and CPA in the presence of Ba^{2+} in murine ureteral smooth muscle were expanded in (Ba) and (Bb). Those data are summarized in (Bc) and (Bd). (C) Ba^{2+}-induced phasic contraction and the enhanced Ba^{2+}-induced contractility by CPA were completely blocked by 1 μM nicardipine (C).

DISCUSSION

The present results can be summarized as follows: (1) Ba^{2+} produced phasic contraction in murine ureteral smooth muscle which was sensitive to nicardipine, and its frequency and amplitude were enhanced by CPA. (2) Ba^{2+}-induced contraction was suppressed by FSK and isoproterenol.

Various K^+ channels which are regulated by Ca^{2+} and ATP have been identified in smooth muscles (Burdyga & Wray, 2005; Hernandez et al, 1997; Knot et al, 1996; Latorre et al, 1989; McCarron & Halpern, 1990; Nelson, 1993; Smith et al, 2002). In general, K^+ channels reduce membrane excitability via activation of its outward K^+ current. Among them, K_{Ca} channel whose gating is activated by $[Ca^{2+}]_i$ is the best known in smooth muscles

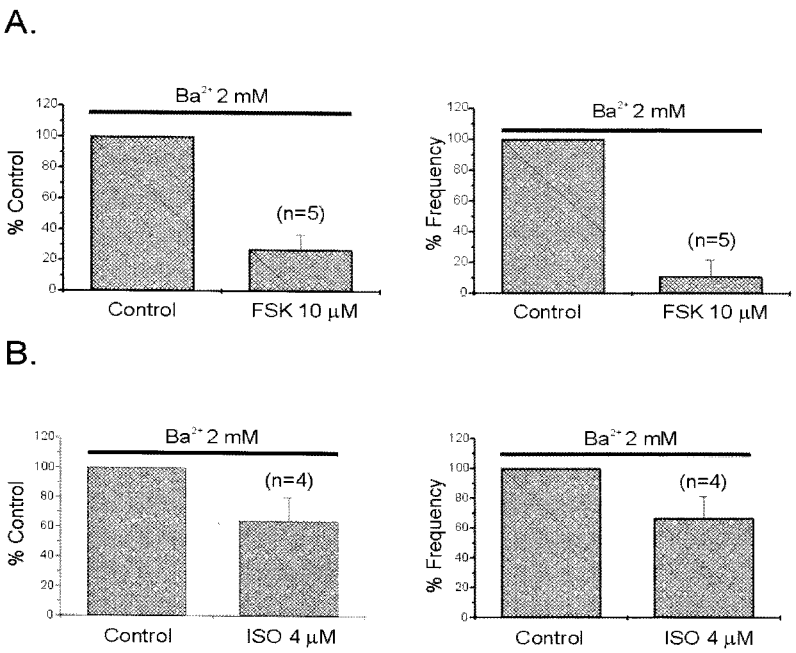

Fig. 4. Effect of forskolin (FSK) and isoproterenol (ISO) on Ba^{2+}-induced contraction in murine ureteral smooth muscle. Regulatory effect of FSK (10 μM) and ISO (4 μM) on Ba^{2+}-induced contraction was studied in (A) and (B). FSK and ISO significantly suppressed the frequency and amplitude of Ba^{2+}-induced contraction ($p<0.05$). Data are summarized in (A) and (B). (A) FSK (10 μM) decreased the amplitude and frequency of Ba^{2+}-induced contraction of 27% and 11% of the control, respectively (n=5). (B) ISO (4 μM) also suppressed the amplitude and frequency to Ba^{2+}-induced contraction to 64% and 67% of the control, respectively (n=5).

(Burdyga & Wray, 2005; Nelson, 1993; Smith et al, 2002; Soares de Moura & deLemos Net, 1996). In fact, K_{Ca} channel in mammalian ureter is also known to be involved in ureteral relaxation and regulation of refractory period. Especially, regulation of refractory period by K_{Ca} channel of ureteral contraction is believed to protect urine reflux and kidney damage (Burdyga & Wray, 2005; Malysz et al, 2004; Soares de Moura & deLemos Net, 1996). However, TEA (1~20 mM) in the present study did not show any significant effect on murine ureteral smooth muscle contractility (data not shown). To date, K_{Ca} channel has been identified in guinea-pig and rat, but not in murine ureter (Smith et al, 2002; Sui & Kao, 1997). Based on this study, murine ureter seems to not express K_{Ca} channel or it may not be a major conductance for regulation of membrane excitability and contraction, and a study at molecular level might be needed to confirm the existence of K_{Ca} channel in murine ureter. Glibenclamide and quinidine did not show any effect on ureteral smooth muscle either (data not shown).

As shown in Fig. 2, 3 and 4, Ba^{2+} produced phasic contraction in murine ureteral smooth muscle, suggesting possible involvement of K_{IR} channel. In general, K_{IR} channels have been reported in many small-diameter cerebral and coronary arteries (Knot et al, 1996; McCarron & Halpern, 1990). In vascular smooth muscle, it is known to provide major conductance around RMP (Knot et al, 1996; McCarron & Halpern, 1990) and believed to play an important role for the regulation of basal tone (Knot et al, 1996). Pharmacologically, K_{IR} channel is known to be blocked by various concentrations of Ba^{2+}, ranging 10 μM~1 mM (Bradley et al, 1999; Kim et al, 2005; Leichtle et al, 2004; Park et al, 2005). In murine smooth muscle, Ba^{2+} (1~2 mM) produced phasic contraction and its effect was not increased by higher concentration of Ba^{2+} (over 2 mM) (Fig. 2A). Therefore, Ba^{2+}-induced contraction of murine ureteral smooth muscle seems to be a result from inhibition of Ba^{2+}-sensitive K_{IR} channel. To date, unfortunately, direct evidence for existence of K_{IR} channel in ureteral smooth muscle cell has not been reported, although one study indicated in guinea-pig ureter an indirect evidence of ureteral spontaneous activity regulated by Ba^{2+} (~1 mM) (Exintaris & Lang, 1999). However, K_{IR} channel is shown to be expressed in ureteric bud in addition to juxtaglomerular cell and nephron epithelia from rat kidney (Braun et al, 2002; Leichtle et al, 2004). Therefore, along with the fact that small-diameter cerebral and coronary arteries diameters of which are similar to that of murine ureter have K_{IR} channels, the existence of K_{IR} channels in ureteric bud suggests a possiblilty of functional expression of K_{IR} channel in murine ureteral smooth muscle and its involvement in regulation of ureteral relaxation, including maintenance of basal tone. However, electrophysiological and molecular studies are required for further confirmation.

Clinically, ureteral obstruction is known to lead to edema and infection of the upper urinary tract (Crowley et al, 1990). Increased intraluminal pressure in the upper urinary tract by obstruction is the major cause of ureteral colic (Moriel et al, 1990). To date, many agents such as spasmolytic (butylscopolamine and calclium channel antagonists) have clinically been used for the relief of ureteral colic and/or promotion of stone passage (Borghi et al, 1994). However, they are generally unsatisfactory regarding safety and efficacy (Borghi et al, 1994). Therefore, understanding regulatory mechanism of ureteral contractility provides

important clinical implication. For the contractility of ureter, acetylcholine (ACh) and norepinephrine (NE) have been known to produce contraction in guinea-pig ureter (Callahan & Creed, 1981). In this case, activation of muscarinic and α-adrenergic receptors and related decrease of cAMP and/or increase of inositol 1,4,5-triphosphate ($InsP_3$) levels have been suggested as candidates (Richards, 1991; Summers et al, 1993). As described in the present study, however, we could not find significant contractility alteration by application of CCh, histamine and serotonin in murine ureter. To date, receptors of murine ureter smooth muscle have not been clarified. Although muscarinic and α-adrenergic receptors were reported in guinea-pig ureter (Wheeler et al, 1995), our limited data suggest the existence of muscarinic receptors in murine ureter with minor importance, since there was some regulatory effect of CCh application only in the presence of high K^+ solution, as shown in Fig. 1C.

As for the relaxation response in ureter, β-adrenergic receptor agonist such as isoproterenol has been regarded as one of the powerful relaxants. β-adrenoceptor was reported to exist in the mammalian ureter, and its activation inhibits ureteral contractility (Weiss et al, 1997). Relaxation by β-adrenergic stimulation suppresses the elevated intraureteral pressure and allows urine flow through previously obstructed region in animal study (Miyatake et al, 2001). Isolated porcine ureter also produces relaxation by isoprenaline, another β-adrenoceptor agonist (Weiss et al, 1997; Danuser et al, 2001). In bladder, β-adrenergic activation is also regarded as the most important physiological stimulation, producing relaxation during the filling and storage phase of the micturition process (Anderson 2004; Yamaguchi, 2002). As shown in Fig. 4B, we also found that isoproterenol suppressed the frequency and amplitude of Ba^{2+}-induced contraction in murine ureteral smooth muscle, and that its inhibitory effect was not observed in the presence of propranonol, a nonspecific β-adrenergic receptor antagonist (data not shown, n=3). In addition, FSK, adenylyl cyclase activator, mimicked the effect of isoproterenol on murine ureteral smooth muscle, since it also suppressed Ba^{2+}-induced phasic contraction (Fig. 4A). Furthermore, inhibitory effects of FSK and isoproterenol were also observed when applied to CPA-induced Ba^{2+} contraction (data not shown). According to the prototypical signaling pathway, FSK and β-adrenergic stimulation increase intracellular cAMP level by activating adenylyl cyclase (Bylund et al, 1994). In fact, inhibitory effect of FSK and β-adrenergic receptor stimulation on the smooth muscle contractility had already been reported (Weiss et al, 1977). Therefore, increase of cAMP level by FSK and β-adrenergic stimulation seems to be a natural event to occur to inhibit murine ureteral smooth muscle contraction, and the down-stream event of signal transduction would be the inhibition of $VDCC_L$ by increased cAMP, as reported recently in guinea-pig (Zhu et al, 2005). Our data (Fig. 2C and Fig. 3C) that Ba^{2+}-induced phasic contraction in murine ureteral smooth muscle was inhibited by nicardipine strongly support this possibility.

In the present study, the effects of CPA and CCCP on murine ureteral smooth muscle were studied. As shown in Fig. 1D, E and F, CPA (10 μM) increased basal tone of ureteral smooth muscle by 0.07 mN (Fig. 1D and F). In smooth muscle, intracellular Ca^{2+} can be released from the SR by either an $InsP_3$-induced Ca^{2+}-release (IICR) or by Ca^{2+} itself, i.e. Ca^{2+}-induced Ca^{2+} release (CICR) (Somlyo and Somlyo, 1994). When the store is unable to take up Ca^{2+} by action of CPA, a inhibitor of Ca^{2+}-ATPase, muscle cell force is increased by increased cytosolic Ca^{2+} concentration. Therefore, the effect of CPA on basal tone of murine ureter might be due to Ca^{2+} released from SR. Tonic increase of $[Ca^{2+}]_i$ by CPA in guinea-pig ureter has already been reported (Burdyga & Wray, 1999). In addition to the increase of basal tone in our murine ureteral smooth muscle, Ba^{2+}-induced phasic contraction was enhanced by CPA (Fig. 3). In guinea-pig ureter, CPA enhances the duration (hence decreased frequency) and spike component of contraction (Burdyga & Wray, 1999). However, in murine ureteral smooth muscle, CPA increased frequency and amplitude of Ba^{2+}-induced phasic contraction. Different effect of CPA on ureteral smooth muscle could be ascribed to sepecies difference and its related various SR regulatory mechanisms (Burdyga et al, 1995). In the present study, the effect of CCCP on ureteral smooth muscle was also studied and as shown in Fig. 1E and 1F, CCCP increased basal tone of ureteral smooth muscle by 0.01 mN. Mitochondria accumulate Ca^{2+} through Ca^{2+} uniporters which is driven by a large negative potential ($-150 \sim -180$ mV) across their inner membrane (Gunter & Peiffer, 1990). Protonophore such as CCCP neutralizes the effect of mitochondrial uniporter by dissipating the mitochondrial Ca^{2+}, hence stopping further accumulation and abolishing mitochondrial Ca^{2+} uptake. Since CCCP is known to increase $[Ca^{2+}]_i$ via these mechanisms, the increase of basal tension by CCCP in murine ureteral smooth muscle also support the increase of $[Ca^{2+}]_i$. However, our data indicate that SR might be functionally more important than mitochondria in the regulation of $[Ca^{2+}]_i$ and motility of murine ureteral smooth muscle, since CPA-induced contraction was greater than that by CCCP.

In summary, our present results showed that Ba^{2+} produced phasic contraction in murine ureteral smooth muscle probably by blocking Ba^{2+}-sensitive K_{IR} channel. The frequency and amplitude of this Ba^{2+}-induced contraction were enhanced by CPA, and suppressed by FSK and isoproterenol which are known to increase intracellular cAMP level. Therefore, these results suggest that the existence of Ba^{2+}-sensitive K_{IR} channel in murine ureteral smooth muscle which is regulated by cAMP pathway.

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