Full Paper

Bladder Contractility Is Mediated by Different K⁺ Channels in the Urothelium and Detrusor Smooth MuscleAneira Gracia Hidayat Santoso¹ and Willmann Liang^{1,*}¹*School of Biological Sciences, College of Science, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551**Received June 16, 2010; Accepted November 3, 2010*

Abstract. The roles played by K⁺ channels in the urothelium (UE) and detrusor smooth muscle (DSM) in regulating agonist-induced bladder contraction is not known at present. Thus, the effects in carbachol (CCh)-induced contraction in UE-intact (+UE) and UE-denuded (–UE) rat detrusor strips pretreated with K⁺-channel blockers were investigated here. The K⁺-channel blockers used were 4-aminopyridine (4-AP), glibenclamide (Glib), iberiotoxin (IbTx), charybdotoxin (ChTx), and apamin. In the absence of K⁺-channel blockers, control CCh-induced contractions were more potent in –UE than +UE strips. Treatment with IbTx and apamin resulted in more potent CCh-induced contractions in +UE strips. In –UE strips, CCh potency was increased by ChTx and Glib, but decreased by 4-AP. Different K⁺ channels in the UE and DSM were thus involved in regulating bladder contractions. Contractile mediatory function of these channels, specific to the UE or DSM, may be potential drug targets in the management of bladder disorders.

Keywords: detrusor smooth muscle, urothelium, contractility, K⁺ channel

Introduction

Urine storage and release are two primary functions of the bladder. Well-mediated bladder contractility is essential to normal bladder function. During the unstimulated state, bladder filling is facilitated by a basal level of spontaneous phasic contractile activity (1). The phasic contractions are mediated by a number of K⁺ channels, including voltage-sensitive K⁺ (K_v), ATP-sensitive K⁺ (K_{ATP}), and Ca²⁺-activated K⁺ channels of different conductances — large (BK), intermediate (IK), and small (SK) (2 – 5). When stimulated, for example, by a muscarinic agonist like carbachol (CCh), bladder contraction is effected (6). On the other hand, stimulation with a β -adrenergic agonist elicits bladder relaxation (6). Growing evidence points to a role of K⁺ channels in β -adrenoceptor-mediated bladder relaxation (7, 8). Direct relaxant effects of K⁺-channel openers, for example, rilimakalim and ZD0947, have also been demonstrated in CCh-precontracted detrusor strips (9, 10). Whether K⁺ channels also mediate agonist-induced bladder contrac-

tion remain to be investigated in detail. It is therefore of interest to examine if K⁺-channel modulation affects CCh-induced bladder contractions, as is the case for Ca²⁺ channels (11).

The dome portion of the bladder wall consists of the urothelium (UE) and detrusor smooth muscle (DSM), both having significant roles in regulating contractility. The DSM contributes the driving force behind contraction and relaxation. The physiological importance of the UE has been recognized recently. It has been postulated that the UE serves as both a sensory and a secretory organ (12 – 14). As a secretory organ, the UE releases a yet unidentifiable substance that suppresses DSM contractility (12, 13). Bladder disorders, as exemplified in overactive bladder, often are manifested by impaired UE function (14, 15). Considering the important contractile mediatory role of the UE, both UE-intact (+UE) and UE-denuded (–UE) detrusor strips were examined here. Contractile responses in +UE strips represent contributions from both the UE and DSM, but only the latter is responsible for responses in –UE strips. CCh-induced contractions in +UE and –UE strips pretreated with various K⁺-channel blockers were compared in this study. The subtypes of K⁺ channels in the UE and DSM involved in bladder contractility were determined. Block-

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ade of BK and SK channels in the UE increased CCh potency, whereas the same effect from blocking K_{ATP} , K_v , and BK channels was independent of the UE.

Materials and Methods

Tissue preparation

All procedures were performed according to rules outlined by the Institutional Animal Care and Use Committee at Nanyang Technological University, Singapore (Project approval No.: ARF SBS/NIE-A 003). Six- to seven-week-old Sprague-Dawley rats of either gender were killed by CO_2 asphyxiation. The whole bladder was harvested as previously described and immediately placed in carbogen-aerated ice-cold Krebs' solution (16). The bladder base, which made up about one third of the bladder, was discarded. Only tissues isolated from the bladder dome (detrusor) were used. The bladder dome was cut open along the lateral sides and the UE was exposed. Fine pins were used to fix the tissue on a petri dish coated with Sylgard® (Dow Corning Corp., Midland, MI, USA). Using a razor blade, four strips measuring 5 mm by 1 mm each were dissected from the detrusor (17). Two of the strips were cut with the longer side parallel to the longitudinal axis of the detrusor and two others, with the longer side parallel to the transverse axis of the detrusor. The longer side of the strip was in line with the direction of contractile force measurement. Both +UE and -UE strips were used. In -UE strips, the UE was carefully excised with fine dissecting scissors as described elsewhere (12, 18). All strips were mounted on a tissue myograph system (Model 800MS; Danish Myo Technology, Aarhus, Denmark) containing carbogen-aerated Krebs' solution at 37°C. Isometric tension was monitored in both transverse and longitudinal directions and recorded using a Powerlab interface and the LabChart software (ADInstruments, Bella Vista, Australia).

Experimental protocol

The detrusor strips were allowed to equilibrate for 30 min with multiple washouts. During the equilibration period, baseline tension was consistently adjusted to 2 ± 0.1 grams. Viability of the strips was tested using carbogen-aerated K^+ -Krebs' solution. Experiments were commenced after an additional 30 min of continuous washout. Cumulative dose-response curves (DRCs) of the muscarinic agonist CCh (from 0.01 to 10 μM in half-log increments) were constructed with and without pretreatment with a selective K^+ -channel blocker for 10 min. Blockers used were 4-aminopyridine (4-AP, 10 mM), glibenclamide (Glib, 10 μM), iberiotoxin (IbTx, 0.1 μM), charybdotoxin (ChTx, 0.1 μM), and apamin (0.01 μM). Thirty minutes of continuous washout was allowed be-

tween the control (i.e., with no K^+ -channel blocker) and blocker-treated DRCs. Each detrusor strip was subject to one K^+ -blocker treatment only to avoid residual drug effects. From a total of 41 rat bladders, 82 transverse and 78 longitudinal detrusor strips were used. The sample sizes (in parentheses) of transverse (Tr) and longitudinal (Lg) strips for the various K^+ -blocker treatments are 4-AP (14 Tr, 12 Lg), Glib (16 Tr, 14 Lg), IbTx (18 Tr, 16 Lg), ChTx (14 Tr, 18 Lg), and apamin (20 Tr, 18 Lg). Half of the strips were +UE, the other half -UE.

Data and statistical analyses

Calculations and statistical analyses were performed using the Prism 4 software (GraphPad Software Inc., La Jolla, CA, USA). The peak tension elicited by each addition of CCh in individual detrusor strips was used in the calculations. Raw values were normalized to dry tissue weights, measured after the experiments, so that weight-insensitive contractile force data could be obtained. Maximal CCh-induced contraction in the -UE strip was considered as 100% contraction in plotting the DRCs. From the raw tension values, the sigmoidal regression equation with Hill slope equals one was used to determine the best-fit DRCs. The equation is as follows: response (%) = $\{ \text{minimal tension} + [(\text{maximal tension} - \text{minimal tension}) / (1 + 10^{\log EC_{50} - x})] \} * 100\%$, where x represents log concentration of the agonist, that is, CCh. The potencies of CCh, expressed as log EC_{50} values, within each K^+ -channel blocker treatment in either contractile direction with and without UE was compared using one-way ANOVA and Tukey's post-hoc test. All data shown in graphs and tables were mean values $\pm$ S.E.M. P values of less than 0.05 ($P < 0.05$) were considered to be statistically different.

Drugs and chemicals

The composition of Krebs' solution was as follows: 119 mM NaCl, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4 , 15 mM $NaHCO_3$, 4.6 mM KCl, 1.5 mM $CaCl_2$, and 11 mM D-glucose. For K^+ -Krebs' solution, no NaCl was added but 124 mM KCl was used instead. All constituents remained the same otherwise. All chemicals and drugs used in this study were purchased from Sigma-Aldrich Co. (Singapore). All drugs were dissolved in Ca^{2+} -free Krebs' solution except 4-AP (in 70% ethanol), Glib (in dimethyl sulfoxide), and apamin (in 0.05 M acetic acid). For drugs not dissolved in Ca^{2+} -free Krebs' solution, a dilution of at least 1000 times from the stock drug solution was performed to prevent nonspecific tissue effects due to the solvents.

Results

Inhibitory effect of the UE in CCh-induced detrusor contractions

Contractions induced by CCh with and without K⁺-blocker pretreatment were examined in both +UE and -UE strips. Figure 1 shows typical raw tension tracings (panel A) and DRCs (panel B) of CCh-induced transverse and longitudinal contractions in the control condition, that is, without any K⁺-blocker pretreatment. At sub-maximal CCh concentrations, contractions were larger in the absence of the UE. Potency of CCh, expressed as log EC₅₀ values, was higher in the -UE strips (Table 1). The

inhibitory role of the UE in mediating detrusor contractions was confirmed here.

Detrusor contractions mediated by BK and SK channels in the UE

IbTx (0.1 μ M) was used to block the BK-channel selectively. A leftward shift of the IbTx-treated DRCs were observed in +UE strips only (Fig. 2: Aa and Ac). No effect of IbTx was observed in the -UE strips as illustrated by the largely overlapping DRCs (Fig. 2: Ab and Ad). Log EC₅₀ values in Table 1 showed that CCh potency was significantly higher in IbTx-treated +UE strips only. Similar results were seen with 0.01 μ M apamin, a selec-

Fig. 1. Comparison of carbachol (CCh)-induced contractions in urothelium-intact (+UE) and urothelium-denuded (-UE) rat detrusor strips. Typical raw tension tracings (A) and dose-response curves (DRCs) (B) of cumulative CCh addition to transverse (Tr) and longitudinal (Lg) strips. Contractile force measurements were made in strips isolated along the transverse and longitudinal axes of the detrusor. For comparative purposes, contractions elicited by 1 and 10 μ M CCh are shown, respectively, by (i) and (ii) (panel A). No intrinsic differences were observed between contractions in Tr and Lg directions. Each point on the DRCs (panel B) represents the mean $\pm$ S.E.M. value from 41 rat bladders (dissected into 82 transverse and 78 longitudinal strips). *** P < 0.0001, significant difference in CCh potency between +UE and -UE.

Table 1. Potency of carbachol (CCh)-induced contractions in urothelium-intact (+UE) and urothelium-denuded (–UE) rat detrusor strips under the treatment of K⁺-channel blockers

		+UE		–UE	
(combined from all experiments)	Control	Tr	–5.19 ± 0.07	Tr	–5.69 ± 0.03***
		Lg	–5.31 ± 0.06	Lg	–5.70 ± 0.03***
		+UE		–UE	
		Before adding blocker (control)	After adding blocker	Before adding blocker (control)	After adding blocker
IbTx (0.1 µM)	Tr	–5.4 ± 0.1	–5.8 ± 0.1 [#]	–5.94 ± 0.06	–6.14 ± 0.06
	Lg	–5.24 ± 0.09	5.70 ± 0.08 ^{###}	–5.94 ± 0.04	–6.15 ± 0.06
Apamin (0.01 µM)	Tr	–5.2 ± 0.1	–5.55 ± 0.08 [#]	–5.96 ± 0.04	–5.90 ± 0.07
	Lg	–5.4 ± 0.1	–5.7 ± 0.1 [#]	–5.83 ± 0.04	–5.97 ± 0.03
Glib (10 µM)	Tr	–4.82 ± 0.08	–5.35 ± 0.07 ^{###}	–5.39 ± 0.05	–5.86 ± 0.06 ⁺⁺⁺
	Lg	–5.2 ± 0.2	–5.71 ± 0.09 ^{##}	–5.52 ± 0.06	–5.87 ± 0.04 ⁺
ChTx (0.1 µM)	Tr	–5.16 ± 0.08	–5.7 ± 0.1 ^{##}	–5.73 ± 0.05	–6.08 ± 0.04 ⁺
	Lg	–5.33 ± 0.06	–5.7 ± 0.1 ^{##}	–5.59 ± 0.04	–5.96 ± 0.04 ⁺⁺
4-AP (10 mM)	Tr	–5.03 ± 0.07	–4.64 ± 0.09 ^{##}	–5.58 ± 0.07	–4.88 ± 0.07 ⁺⁺⁺
	Lg	–5.4 ± 0.1	–4.9 ± 0.1 [#]	–5.59 ± 0.06	–4.9 ± 0.1 ⁺⁺

Blockers used were as follows: 4-aminopyridine (4-AP), glibenclamide (Glib), iberiotoxin (IbTx), charbydotoxin (ChTx), and apamin. Contractile force measurements of detrusor strips were performed in both transverse (Tr) and longitudinal (Lg) directions. Potency of CCh is expressed as log EC₅₀. Values shown are mean ± S.E.M. *** denotes $P < 0.0001$ between control values of +UE and –UE strips. Statistical significances between blocker-treated and control values in +UE strips are denoted by [#] (for $P < 0.05$), ^{##} (for $P < 0.01$), and ^{###} (for $P < 0.0001$). Statistical significances between blocker-treated and control values in –UE strips are denoted by ⁺ (for $P < 0.05$), ⁺⁺ (for $P < 0.01$), and ⁺⁺⁺ (for $P < 0.0001$).

tive SK-channel blocker. In the presence of apamin, +UE strips showed larger contractions at submaximal CCh concentrations (Fig. 2: Ba and Bc), while the contractility of –UE strips was unaltered (Fig. 2: Bb and Bd). CCh potency was significantly higher in apamin-treated +UE strips only (Table 1). The data from IbTx and apamin treatments suggested that both BK and SK channels in the UE but not DSM mediated CCh-induced detrusor contractions.

Detrusor contractions mediated by K_v, K_{ATP}, and IK channels in the DSM

Unlike BK and SK channels, the effect on contractility from IK-channel blockade by 0.1 µM ChTx was not dependent on the UE. CCh potency was higher in ChTx-treated contractions in both +UE and –UE strips. In –UE strips, DRCs were shifted to the left under ChTx treatment (Fig. 3: Ab and Ad). The UE (Fig. 3: Aa and Ac) did not provide any influence on the ChTx-mediated effects seen in –UE strips. Results were similar with 0.1 µM Glib treatment, in which the K_{ATP} channel was blocked. In the presence of Glib, higher CCh potency (Table 1) was demonstrated in both +UE (Figure 3: Ba and Bc) and –UE strips (Fig. 3: Bb and Bd). Instead of a potentiating effect, treatment with the selective K_v-

channel blocker 4-AP (10 mM) resulted in diminished contractility. A rightward shift of the DRCs was seen in both +UE (Fig. 3: Ca and Cc) and –UE strips (Fig. 3: Cb and Cd). Potency of CCh was lower in the presence of 4-AP in both +UE and –UE strips (Table 1). Altogether, blockade of IK, K_{ATP}, and K_v channels revealed mediatory effects on contractions that were independent of the UE.

Discussion

The vast majority of studies using K⁺-channel blockers examined phasic contractile activity in the detrusor. This underlies the functional importance of K⁺ channels in mediating spontaneous phasic contractions in the bladder (1). On the contrary, the role of K⁺ channels in mediating agonist-induced bladder contractions is poorly understood. The current study was thus conducted to investigate the effects of K⁺-channel blockade in CCh-induced contractions in the detrusor. Since the UE has been suggested to play an active role in mediating DSM contractility, force measurements of both +UE and –UE detrusor strips were made in this study. Although differential transverse and longitudinal detrusor strip contractility has been documented using various chemicals (e.g.,

Fig. 2. Effects of urothelium-dependent K⁺-channel blockade in the urothelium in CCh-induced contractions. Measurements were made in both transverse (Tr) and longitudinal (Lg) directions. Detrusor strips were pretreated with iberiotoxin (IbTx, 0.1 μ M) (A) or apamin (0.01 μ M) (B) for 10 min prior to CCh addition. Treatment with either IbTx or apamin resulted in higher CCh potency in +UE strips only (panels Aa, Ac, Ba, Bc). The same K⁺-channel blockers had no effect on contractions in -UE strips (panels Ab, Ad, Bb, Bd). Each point on the DRCs represents the mean $\pm$ S.E.M. values from experiments of the particular K⁺-channel blocker. The number of transverse/longitudinal strips used was (in parentheses) as follows: IbTx (18/16) and apamin (20/18). Statistical significances of CCh potency in +UE strips are denoted by # P < 0.05 and ### P < 0.0001 between K⁺-channel blocker-treated and control values.

isoprenaline, ATP) and physical (e.g., fatigue recovery, passive stretching) stimuli (17–21), such phenomenon was not observed here under K⁺-channel blocker treatments.

Various subtypes of K⁺ channels, including K_v, K_{ATP}, and Ca²⁺-activated K⁺ channels of different conductances: BK, IK, and SK are found in the bladder. Using -UE detrusor strips, K⁺-channel function in DSM contractility has been investigated by many. Phasic contractions in the detrusor are under negative influences of K_v, BK, IK, and SK channels (2, 4, 14). Functional relationship between

K⁺ channels and β -adrenoceptor-mediated detrusor relaxations have also been studied (7, 8, 22). Information on K⁺ channel-mediating effects in detrusor contractions is relatively scarce by comparison. By far the only K⁺ channel subtype studied was the K_{ATP} channel, the opening of which induced relaxation in KCl- and CCh-precontracted detrusor strips (9, 10). The role of the K_{ATP} channel in CCh-induced contractions alone was not investigated in those studies. It was demonstrated here that K_{ATP}-channel blockade increased CCh potency regardless of the UE. This finding was in line with that by

Fig. 3. Effects of urothelium-independent K^+ -channel blockade in CCh-induced contractions. Measurements were made in both transverse (Tr) and longitudinal (Lg) directions. Detrusor strips were pretreated with charybdotoxin (ChTx, $0.1 \mu\text{M}$) (A), glibenclamide (Glib, $10 \mu\text{M}$) (B), and 4-aminopyridine (4-AP, 10 mM) (C) for 10 min. Treatment with either ChTx or Glib resulted in higher CCh potency in both +UE (panels Aa, Ac, Ba, Bc) and -UE strips (panels Ab, Ad, Bb, Bd). CCh potency in both +UE (panels Ca, Cc) and -UE strips (panels Cb, Cd) was lower after treatment with 4-AP. Each point on the DRCs represents the mean $\pm$ S.E.M. values from experiments of the particular K^+ channel blocker. The number of transverse/longitudinal strips used was (in parentheses) as follows: Glib (16/14), ChTx (14/18), and 4-AP (14/12). Statistical significances of CCh potency are denoted by $\#P < 0.05$, $##P < 0.01$, and $###P < 0.0001$ between K^+ -channel blocker-treated and control values in +UE strips and by $+P < 0.05$, $++P < 0.01$, and $+++P < 0.0001$ between K^+ -channel blocker-treated and control values in -UE strips.

Wuest et al., which showed that relaxation induced by rilimakalim (an K_{ATP}-channel opener) was not dependent on the UE as well (9). Besides the K_{ATP} channel, K_v and IK channels were also found to mediate CCh-induced contractions independent of the UE. The physiological function of K_v and IK channels in the bladder has not been investigated, although the latter may function like other Ca²⁺-activated K⁺ channels in facilitating bladder relaxation (23). It is only known that K_v-channel blockade enhanced phasic contractile activity (2, 17). In this study, we added that K_v-channel blockade diminished agonist-stimulated contractions, an effect opposite to that in unstimulated phasic contractions. By studying contractility under various physical or chemical challenges, K_v-channel function in bladder physiology will be better understood.

Among the K⁺-channel blockers used, both IbTx and apamin treatments resulted in more potent CCh contractions in only +UE strips. The present evidence thus suggests BK and SK channels in the UE having a unique role in mediating CCh-induced detrusor contractions. Expression of BK and SK channels in the UE has been demonstrated (23, 24). Others have studied the function of BK and SK channels in +UE and -UE phasic contractions in the detrusor (2, 4, 23, 25) and reached the same conclusion about the inhibitory role of these channels. In contrast, there was very little information about UE, BK, and SK channels in mediating agonist-stimulated detrusor contractions. Hawthorn et al. reported a lack of effect from UE, BK, and SK channel blockade in CCh contractions in the pig detrusor (12). The opposite was observed in this study using rat detrusor. Such discrepancy may be attributed to species difference, as has been exemplified in K_{ATP} channel-inhibited contractility between pig and human (and mouse) detrusor (9). In order to determine the role of BK and SK channels in the UE, more comparative studies in different species of animals will be of considerable importance.

In summary, this study examined UE-dependent and UE-independent CCh-induced contractions in the detrusor under various K⁺ channel-blocker treatments. The active role of the UE in regulating bladder contractility was confirmed. Different subtypes of K⁺ channels in the UE and DSM were involved in mediating the contractions. In the UE, blockade of BK and SK channels resulted in more potent CCh responses. Effects on CCh-induced contractions due to blockade of IK, K_{ATP}, and K_v channels were independent of the UE. It was demonstrated for the first time that urothelial K⁺ channels have important roles in agonist-induced bladder contractions. These K⁺ channels could potentially serve as drug targets in restoring normal bladder contractile function in diseased states. Findings from this study may therefore

promote drug development in the management of bladder disorders associated with detrusor overactivity (15, 26).

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