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Differential Effects of K^+ Channel Blockers on Phasic Contractility of Transverse and Longitudinal Rat Detrusor Strips

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Abstract

Spontaneous phasic contractions of detrusor smooth muscle are pivotal to the normal bladder filling process. The role of K^+ channels in mediating phasic contractions has been investigated on different occasions, but only in detrusor strips isolated longitudinally. In this study, the effects of individual K^+ blockers were examined in both transverse and longitudinal detrusor strips. Detrusor strips were isolated transversely and longitudinally from young adult rat bladders. Tension before and after the introduction of K^+ channel blockers was measured using a myograph. Phasic activity was determined by calculating the integral of tension fluctuations. Phasic activity of transverse strips was increased under tetraethylammonium chloride (TEA), 4-aminopyridine (4-AP) and iberiotoxin (IbTx) treatments. Longitudinal phasic activity was increased under charybdotoxin (ChTx) treatment. Neither glibenclamide (Glib) nor apamin treatment elicited any significant effect in both transverse and longitudinal phasic activity. The results indicated that phasic activity was mediated differently depending on the contractile direction. Data from this study reiterate that in addition to the conventional longitudinal direction, the transverse direction also presents significance when examining the contractility of a sac-like organ like the bladder.

Keywords: Detrusor Smooth Muscle, Contractility, K^+ Channel, Transverse, Longitudinal

1. Introduction

Urine release and storage are major functions of the urinary bladder. Forceful contractions of the detrusor smooth muscle are essential for urine release. During bladder filling, detrusor wall tension and intravesical pressure fluctuate to adjust to the changing urine volume [1]. The ever changing contractility of the detrusor smooth muscle contributes to spontaneous phasic contractions frequently seen in whole bladder or isolated strip experiments. Regulation of phasic contractions can be of several origins, from nerves, the urothelium or within the smooth muscle [2]. The importance of phasic activity in the bladder is implicated in various disease conditions [2-4]. Indeed many groups have investigated one aspect or another of phasic contractions, both in normal and diseased bladders. One group of molecular candidates believed to mediate phasic contractions is the K^+ channels. Several groups have studied the effects of K^+ channel blockers and reached variable findings attributable to differences between species and experimental conditions [5-9]. Although not as extensively used as other animals, the rat remains a valuable model for studying bladder physiology. Diseased bladder models have been established in the rat [4,10]. Findings from the normal rat bladder therefore provide good references to identify diseased-induced changes in disease models. The vast majority of literature on bladder contractility of the rat (or other animals) reported on longitudinal contractions only, irrespective of contractile differences between transverse and longitudinal directions [11-15]. These differences point to the importance in considering contractions in more than one direction. Thus, this study is conducted to identify differential modulation by K^+

channels in detrusor phasic contractions between transverse and longitudinal directions.

2. Materials and Methods

2.1. 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. Forty-nine rats were used in total, from which 40 transverse and 30 longitudinal strips were isolated (see below). The whole bladder was harvested as previously described and immediately placed in carbogen-aerated ice-cold Krebs' solution [11]. 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 urothelium was exposed. Fine pins were used to fix the tissue on a Sylgard®-coated petri dish. Using a razor blade, two strips measuring 5 mm by 1 mm each were dissected from the detrusor. One strip was cut with the longer side parallel to the longitudinal axis of the detrusor. The other strip was cut 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 measurements as previously done [12]. The urothelium was kept intact. All strips were mounted on a tissue myograph system (Danish Myo Technology Model 800MS, Aarhus, Denmark) containing 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).

2.2. Experimental Protocol

The detrusor strips were allowed to equilibrate for 30 minutes with multiple washouts at 2 g of resting tension. The viability of the strips was tested using K^+-Krebs' solution bubbled with a mixture of 95% oxygen and 5% carbon dioxide. After another 30 minutes of continuous washout, a K^+ channel blocker was added. The effective concentration of each K^+ channel blocker in rat detrusor strips was pre-determined in previous studies [12,13]. Blockers used were as follows (concentrations in parentheses): tetraethylammonium chloride (TEA, 10 mM), 4-aminopyridine (4-AP, 3 mM), glibenclamide (Glib, 0.1 μM), iberiotoxin (IbTx, 0.1 μM), charybdotoxin (ChTx, 0.1 μM) and apamin (0.1 μM). The incubation period of each K^+ channel blocker was 10 minutes.

2.3. Drugs and Chemicals

The composition of Krebs' solution was as follows (in mM): NaCl (119), $MgCl_2$ (1.2), NaH_2PO_4 (1.2), $NaHCO_3$ (15), KCl (4.6), $CaCl_2$ (1.5), Glucose (11). 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, Singapore). All drugs were dissolved in Ca^{2+}-free Krebs' solution except 4-AP (in 70% ethanol), Glib (in dimethyl sulfonide) and apamin (in 0.05 M acetic acid). For drugs not dissolved in Ca^{2+}-free Krebs' solution, a maximum of 1-to-1000 dilution from the stock drug solution was performed to prevent nonspecific tissue effects due to the solvents.

2.4. Data and Statistical Analysis

Sample tracings were shown in pairs, consisting of control and K^+ blocker-treated readings from the same detrusor strip. Fluctuations in tension (ΔF) were expressed as a percentage of the maximal contraction (F) to K^+-Krebs' solution. According to the literature, the integral under the tension recording curve could give an indication of the amount of phasic activity [11,16]. In this study, phasic activity was quantified this way for a 2-minute period before (*i.e.* control) and after the addition of each K^+ blocker. For K^+ channel blocker-mediated phasic activity, readings were taken starting at 10 minutes after the addition of the blocker. Phasic activity in the presence of the K^+ channel blocker was expressed as a percent change from the control phasic activity. Statistical analysis was done using the Prism 4 software (GraphPad Software Inc., La Jolla, CA, USA). Student's t-test was used to determine if the percent change in phasic activity was significantly deviated from zero, which was the control level by definition. The difference between the percent change in transverse and longitudinal phasic activity was also determined by Student's t-test. All data shown in graphs were mean ± SEM. P values of less than 0.05 ($P < 0.05$) were considered to be statistically different.

3. Results

3.1. Transverse Phasic Activity Was More Sensitive to Blockade of Voltage-Sensitive K^+ Channels

Tetraethylammonium chloride (TEA) is a nonselective

blocker of voltage-sensitive K^+ (K_v), ATP-sensitive K^+ (K_{ATP}) and large-conductance Ca^{2+}-activated K^+ (BK) channels. **Figure 1(a)** shows sample tracings of phasic activity before (*i.e.* control) and after adding the K^+ channel blockers. In both transverse and longitudinal directions, phasic activity was significantly increased by 10 mM TEA treatment (**Figure 1(b)**). Transverse phasic activity was increased by 110% ± 20%, compared to a 60% ± 10% increase in longitudinal phasic activity ($P < 0.05$). Since TEA could act at more than one type of K^+ channels, namely K_v, K_{ATP} and BK channels, selective blockade of K_v and K_{ATP} channels, by 4-aminopyridine (4-AP) and glibenclamide (Glib) respectively, was examined next. In the presence of 3 mM 4-AP, transverse phasic activity was significantly increased (by 50% ± 20%) whereas that of longitudinal remained unchanged from control (10% ± 10%) (**Figure 1(b)**). Similar to TEA treatment, 4-AP elicited a greater effect in transverse than longitudinal phasic activity ($P < 0.05$). Under 0.1 μM Glib treatment, there was a small but not significant decrease in both transverse and longitudinal phasic activity, respectively, by 30% ± 10% and 20% ± 20% (**Figure 1(b)**). The function of K_{ATP} channels in mediating phasic activity was not implicated, whereas that of K_v channels was demonstrated in the transverse contractile direction.

3.2. Blockade of Ca^{2+}-Activated K^+ Channels Revealed Differential Effects in Transverse and Longitudinal Phasic Activity

Figure 2(a) shows sample tracings of phasic activity before and after adding the various Ca^{2+}-activated K^+ channel blockers. Aside from K_v and K_{ATP} channels, BK channels are also blocked by TEA. Iberiotoxin (IbTx) was used here to block BK channel activity selectively. Only transverse phasic activity was significantly increased (by 80% ± 20% from the control level) under 0.1 μM IbTx treatment (**Figure 2(b)**). As with TEA and 4-AP, IbTx also elicited a greater stimulatory effect in transverse than longitudinal phasic activity ($P < 0.05$). Charybdotoxin (ChTx) is a blocker of intermediate-conductance Ca^{2+}-activated K^+ (IK) channels. In the presence of 0.1 μM ChTx, longitudinal phasic activity was significantly increased from the control level by 60% ± 20% (**Figure 2(b)**). The greater effect elicited by ChTx in longitudinal phasic activity was approaching statistical significance ($P = 0.0765$). After adding 0.1 μM apamin, a selective blocker of small-conductance Ca^{2+}-activated K^+ (SK) channels, both transverse and longitudinal phasic activity was only modestly suppressed, by 20% ± 10% and 20% ± 9% (**Figure 2(b)**). The findings here suggested that both BK and IK channels mediated phasic activity, but did so distinctly according to the contractile direction.

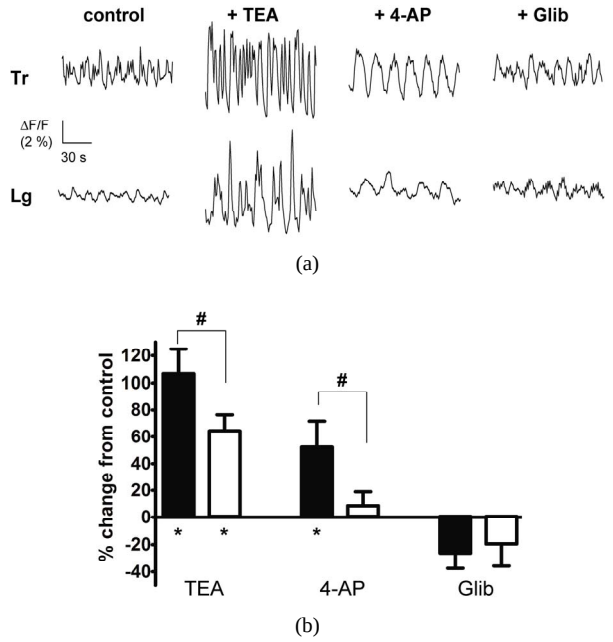

Figure 1. Transverse and longitudinal phasic contractions under the influence of nonselective and selective voltage-sensitive K^+ (K_v) and ATP-sensitive K^+ (K_{ATP}) channel blockers. (a) Representative tracings of transverse (Tr) and longitudinal (Lg) phasic contractile activity before (*i.e.* control) and after treatment with 10 mM tetraethylammonium chloride (TEA) (Tr: n = 4; Lg: n = 4), 3 mM 4-amino- pyridine (4-AP) (Tr: n = 8; Lg: n = 5) or 0.1 μM glibenclamide (Glib) (Tr: n = 6; Lg: n = 5); (b) Percent change in transverse (solid columns) and longitudinal (open columns) phasic activity from the control level. Significantly higher phasic activity was present in the transverse direction under TEA and 4-AP treatments. *denotes P < 0.05 vs. control level at 0%. #denotes P < 0.05 between transverse and longitudinal.

4. Discussion

The role of K^+ channels in mediating spontaneous phasic contractions in the detrusor have been studied by different groups over the recent years. Both urothelium-intact and -denuded detrusor tissues from the bladder of different species have been used, often yielding conflicting results [5,6,8,12,13]. Thus, generalizations, if any, about K^+-channel-mediated phasic contractions in the detrusor are yet to be made. Using selective K^+ channel blockers at effective concentrations previously established, we hereby examined phasic contractile activity in both transverse and longitudinal directions using normal adult rat urothelium-intact detrusor strips. Despite anatomical differences between human and rat detrusor, the latter is still of value due to the numerous established bladder diseased models in the rat. Examples include the overact-

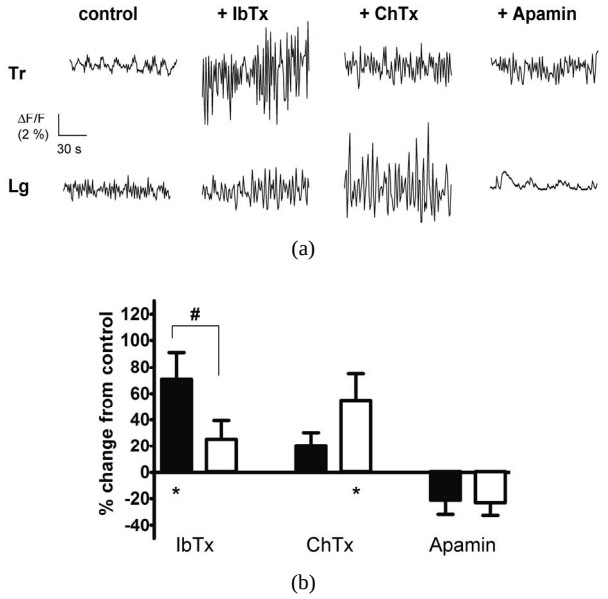

Figure 2. Transverse and longitudinal phasic contractions under the influence of selective Ca^{2+}-activated K^+ channel blockers. (a) Representative tracings of transverse (Tr) and longitudinal (Lg) phasic contractile activity before (*i.e.* control) and after treatment with 0.1 μM iberiotoxin (IbTx) (Tr: n = 8; Lg: n = 6), 0.1 μM charybdotoxin (ChTx) (Tr: n = 8; Lg: n = 5) or 0.1 μM apamin (Tr: n = 6; Lg: n = 5); (b) Percent change in transverse (solid columns) and longitudinal (open columns) phasic activity from the control level. Transverse phasic activity was increased under IbTx treatment whereas that of longitudinal was increased in the presence of ChTx. *denotes $P < 0.05$ vs. control level at 0%. $^{\#}$denotes $P < 0.05$ between transverse and longitudinal.

tive bladder model in spontaneously hypertensive rats and the rat bladder outlet obstruction model [4,10]. Findings in this study would lay the groundwork for comparisons with the contractility of diseased rat detrusor, which would in turn give insights into altered physiology in the human bladder in the future.

Whereas most other studies measured detrusor strip contractility in the longitudinal direction, we also considered transverse contractions in our experiments. Differences in contractile directions, *i.e.* transverse vs. longitudinal, in the detrusor have been reported under different experimental conditions [11-15]. Choice of species and method of tissue preparation (*i.e.* urothelium-intact vs. denuded tissues, or isolated strips vs. whole bladders) apparently resulted in contrasting findings by different groups. Nevertheless we demonstrated in the present study that selected K^+ channel blocker treatments could reveal directional phasic contractile differences. These differences should be considered when attempting to draw conclusions from contractile data of a single direction only.

The stimulatory effect of various K^+ channel blockers in phasic contractions has been well documented, indicating the role of K^+ channels in mediating the tension fluctuations [1]. In the basal or unstimulated state, K_{ATP} and SK channels had no significant functional role in longitudinal phasic activity of urothelium-intact detrusor [5,6], findings supported by our data as well. We further showed that transverse phasic activity was equally unaffected by K_{ATP} and SK channel blockade. The distinctive effects in transverse and longitudinal phasic activity were demonstrated by blocking K_v, BK and IK channels. The nonselective K^+ channel blocker TEA enhanced phasic activity in both transverse and longitudinal directions. The effect in transverse phasic activity was greater due to possibly larger contribution by K_v and BK channels, both of which blocked by TEA, in this direction. This was supported by the results of 4-AP and IbTx treatments where transverse phasic activity was significantly higher than the control level. The more prevalent transverse phasic activity seen under 4-AP treatment has been demonstrated elsewhere [12]. For IbTx, others have shown different results depending on tissue origins and preparation. In the longitudinal guinea-pig detrusor, phasic activity was increased under IbTx treatment [8]. This was in contrast to the whole rat detrusor where no change in phasic activity was detected [9]. It is possible that phasic activity in individual smooth muscle bundles may not influence intravesical pressure in the whole bladder to a great extent. Nevertheless, an intrinsic function in the greater sensitivity of transverse phasic activity toward BK, and also K_v channel blockade may be implicated. Although ChTx could block BK channels, its use as an IK channel blocker has been documented. In the longitudinal but not transverse direction, phasic activity was enhanced under ChTx treatment, again suggesting direction-dependent differences in the regulation of phasic contractions.

Spontaneous detrusor phasic contractions occur during bladder filling to allow maintenance of bladder shape and wall tension without drastically increasing intravesical pressure [1]. This prevents immature voiding as well as ensures efficient micturition when necessary. Other than K^+ channels as demonstrated in this and other studies, Ca^{2+} channels [3], gap junctions [3] and the urothelium [5,17,18] also play a part in mediating phasic contractions. The role of the mucosal layer (including the urothelium and myofibroblasts) is of particular interest especially in disease conditions [2]. The use of normal rat detrusor here nevertheless serves as a starting point to examine disease-induced changes in bladders from established rat models. Regardless of species and experimental variations, the urothelium is believed to be an active participant of normal and diseased bladder physiology. Studies comparing the role of K^+ channels in me-

diating urothelium-dependent and urothelium-independent detrusor phasic contractions may be useful. It is not possible to isolate the effects of the urothelium from those of the smooth muscle if the intravesical pressure of the whole bladder is measured, although this method resembles *in vivo* physiology more closely.

In summary, the role of individual K^+ channel blockers in mediating phasic activity in the detrusor was examined. In measuring phasic contractions in two directions, transversely and longidutinally, differential sensitivity to the K^+ channel blockers was demonstrated. Phasic activity in the transverse direction could be distinguished from that in the longitudinal direction by using selective blockers of K_v, BK and IK channels. The discovery of differential phasic activity highlights the potential importance in considering the physiological function of contractility in more than one direction.

5. Acknowledgements

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