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Phasic activity of urinary bladder smooth muscle in the streptozotocin-induced diabetic rat: Effect of potassium channel modulators

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Abstract

Myogenic mechanisms, whereby there are fundamental changes to the electromechanical properties of detrusor muscle, have been proposed as a cause of detrusor overactivity, and thus may involve one of the key regulators of UBSM electrical properties, potassium (K) channels. In this study, we investigated the effect of potassium channel modulators in regulating the phasic activity (PA) of bladder strips from the streptozotocin (STZ)–induced diabetic rat. Strips of bladder, isolated from male rats 1-week following STZ (65mg/kg, i.p.) administration and age-matched controls, were mounted under 2g tension in tissue baths containing Krebs-bicarbonate solution at 37°C. The effects of K channel modulators was investigated on resting basal tension or on PA induced by low concentrations of the muscarinic agonist carbachol (CCH) (0.5µM). Activation of large conductance calcium activated K (BK) by NS1619 had a minor effect on CCH-induced PA of bladder strips from both control and diabetic animals, and significantly inhibited amplitude in a similar fashion only at 30µM. Activation of ATP-sensitive K (K_{ATP}) channels by cromakalim (3µM and 10µM) did not affect amplitude, but inhibited the frequency of CCh-induced PA of bladder strips in a similar manner in both groups of tissues, although strips from diabetic rats demonstrated a trend towards being less sensitive to the effects of cromakalim. The BK channel blocker iberiotoxin (100nM) was able to induce PA in resting tissues, with diabetic tissues demonstrating significantly enhanced PA compared to controls. In contrast inhibition of SK and K_{ATP} channels did not induce PA in resting tissues. In conclusion opening of BK and KATP channels produced similar inhibitory effects on CCH-induced PA in control and diabetic rat bladder strips. Conversely, blockade of BK channels, but not K_{ATP} or SK channels, induced significantly greater PA in bladder strips from diabetic animals compared to controls. Overall results suggest

altered BK and K_{ATP} channel function in the diabetic rat bladder, which may contribute to bladder dysfunction in this model. 313 words

Keywords: bladder, phasic contractions, potassium channel modulation, streptozotocin-induced diabetes

Abbreviations:

1. Introduction

Urinary bladder smooth muscle (UBSM) exhibits spontaneous action potentials that are associated with phasic activity (PA) in this tissue (Heppner *et al*, 1997; Brading, 2006). Ca^{2+} entry through voltage dependent Ca^{2+} channels (VDCCs) is responsible for the upstroke of the action potentials whilst the repolarisation phase is mediated by the activity of large conductance Ca^{2+} activated K^+ (BK) channels (Heppner *et al*, 1997). The resting membrane potential of UBSM is modulated collectively by BK, ATP sensitive K^+ (K_{ATP}) and probably K_V channels (Heppner *et al*, 1997; Petkov *et al*, 2001; Thorneloe & Nelson, 2003). The activation of small conductance Ca^{2+} activated K^+ (SK) channels is also important for generation of long-lasting hyperpolarisation termed the slow afterhyperpolarisation (sAHP) (Fujii *et al*, 1990).

Since K channels play a vital role in mediating UBSM cell membrane potential, changes in K channel function can effect the repolarisation of UBSM cells after a transient depolarisation. These changes may subsequently result in enhanced PA, which has been associated with detrusor overactivity (DO), and may lead to urgency and urinary incontinence (Werner *et al*, 2007; Brown *et al*, 2008; Chang et al, 2010; Kita et al, 2010). Thus opening of K channels may be an attractive way of treating overactive detrusor, by eliminating undesired bladder contractions and increasing the UBSM stability (Gopalakrishnan & Shieh, 2004). This area has become a focus of research interest, with many studies investigating the role of BK, SK and K_{ATP} channels in mediation of PA of the bladder detrusor muscle of various species (Herrera *et al*, 2000; Petkov *et al*, 2001; Gopalakrishnan *et al*, 2002; Herrera *et al*, 2003; Fey *et al*, 2003; Malysz *et al*, 2004; Ng *et al*, 2006; Chang et al, 2010),

In the streptozotocin (STZ)-induced diabetic rat, which has commonly been used as a model of bladder dysfunction (Steers, 1994; Pitre et al, 2002; Stevens et al, 2006; Wang et al, 2009), bladder strips show increased PA (Tammela et al, 1994; Nakahara et al, 2004, and Stevens et al, 2006), although the underlying mechanism is unknown. Nakahara et al (2004_ investigated the role of BK and SK channels in modulating this PA in the detrusor of the diabetic rat. These authors demonstrated that although diabetes increased basal UBSM mechanical activity, the main negative feedback system mediated by BK channels remained preserved (Nakahara et al, 2004). Apart from this report, there are few studies investigating the role of K channels in mediating PA in the detrusor from the STZ-diabetic rat model. Therefore, the aim of the present study was to investigate the effect of various K channel in regulating the PA of the STZ–induced diabetic rat bladder and age-matched controls.

2. Methods

2.1. Animals and streptozotocin treatment

Male Wistar-Hannover rats (Charles River, UK), approximately 250g body weight, were used in this study. Diabetes was induced by a single intraperitoneal injection of streptozotocin (65mg kg^{-1} body weight; dissolved in 0.01M citrate buffer, pH 4.5). Following induction of diabetes animals were kept for a period of 1-week, with free access to food and water. At sacrifice, animals with blood glucose levels greater than 15mM were considered diabetic. Control animals were age-matched. All procedures were performed in accordance with UK current Home Office Project and Personal licences (PPL 40/4778 & 40/3011).

2.2. Tissue preparation and isometric tension recordings

The bladders were removed from 1-week diabetic animals and their age-matched controls. A longitudinal incision was performed through the bladder from the base to the dome and the bladder was opened up to form a flat sheet. The base and the top of the dome were carefully removed and 3-4 longitudinal strips, depending on bladder size, measuring 2-4 x 6-12mm were then cut from the bladder body. Tissues were suspended in 15ml organ baths containing Krebs-bicarbonate solution (118.3mM NaCl, 11.7mM D-Glucose, 24.9mM $NaHCO_3$, 4.7mM KCl, 1.15mM $MgSO_4$, 1.15mM KH_2PO_4 and 1.9 mM $CaCl_2$) including 5µM indomethacin (a cyclooxygenase inhibitor), maintained at 37ºC and gassed with 95% O_2 and 5% CO_2. The strips were placed under 2g of tension and left to equilibrate for 60 min. and the tension developed by the tissues was measured using isometric force transducers (Pioden Controls Ltd, UK) connected to a Powerlab data acquisition system using 'Chart' software (ADInstruments, UK).

2.3. Experimental design

2.3.1. Effect of BK and K_{ATP} channel openers on PA

Following equilibration, tissues were stimulated with 0.5μM of the muscarinic receptor agonist, carbachol to induce PA, as previously described by Ng *et al* [3]. In the continued presence of CCH, increasing cumulative concentrations of the BK channel opener NS1619 (hydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2Hbenzimidazol-2-one) (1-30μM) or K_{ATP} channel opener, cromakalim (0.1-10μM) were added to the tissues and the effects of these drugs on the amplitude and the frequency of PA was assessed. The tissues were incubated with each concentration of K channel opener for a period of 10-15mins. The effect of the vehicle, DMSO (dimethyl sulfoxide) (%), was also examined on the PA in parallel experiments to account for any time- or vehicle-dependent changes in the PA.

2.3.2. Effect of BK, SK and K_{ATP} channel blockers on PA

After assessing the effect of K channel openers, bladder strips were washed for 30mins with fresh Krebs bicarbonate solution. Following return to resting tension, tissues were exposed to increasing concentrations of the BK channel blocker IBTX (iberiotoxin) (0.01-0.1μM), the SK channel blocker apamin (0.01-0.1μM) or the K_{ATP} channel blocker glibenclamide (1-10μM). Tissues were incubated with each concentration of K channel blocker for a period of 10-15mins.

2.4. Data Analysis

The last 5mins period with each concentration of each drug was used to calculate the mean amplitude and the frequency of the PA. To calculate the amplitude and the frequency of PA, a slightly modified method to that proposed by Imai *et al* (2001) was used to define a single

phasic contraction event. Firstly, the maximum amplitude of contractile activity over a 5mins period was calculated in control tissues. Any contractions over and above 30% of this were considered as single phasic contractions and counted for calculation of the frequency. The same 30% threshold was also used to determine the frequency of PA in diabetic tissues. Data is expressed as mean ± SEM.

Statistical analysis was performed using repeated measures *ANOVA* followed by Dunnett's post hoc test for intra-tissue variations and unpaired Student's *t*-test for inter-tissue variations. $P<0.05$ was considered significant.

2.5. Drugs

Carbachol, dimethyl sulfoxide, apamin, glibenclamide, cromakalim and NS1619 were obtained from Sigma-Aldrich (Dorset, UK). Iberiotoxin was obtained from Alomone labs (Jerusalem, Israel).

3. Results

3.1. Development of PA

CCH (0.5μM) induced PA in bladder strips from control and diabetic rats (Figure 1). The amplitude of PA in tissues from 1-week diabetic animals (0.14±0.01 g/mg tissue, n=31) was significantly (P<0.001) greater than in the age-matched control tissues (0.07±0.01 g/mg tissue, n=24). In contrast to amplitude, the frequency of PA in tissues from 1-week diabetic animals (32.19±1.54 events in 5mins) was significantly (P<0.001) less than in the age-matched control tissues (62.30±5.6 events in 5mins).

3.2. Effect of NS1619 on phasic contractions

NS1619 had a minimal effect on PA in bladder strips from 1-week diabetic animals and their age-matched control (non-diabetic) group. A significant inhibition of the amplitude of CCH-induced PA was observed only at the highest concentration (30μM) (Figures 2 & 3). NS1619 did not affect the frequency of PA at any of the concentrations examined (Figures 2 & 3) in either tissue group.

When data was normalised to show the percentage change in the amplitude of the PA in the presence of the maximal concentration of 30μM NS1619 relative to PA in the absence of the opener (i.e. in the presence of 0.5μM CCH only), the inhibition was similar between age-matched control (28.76±4.16%) and diabetic (24.35± 4.2%) tissues. The vehicle, DMSO, did not produce any significant effects on the amplitude or the frequency of PA in either control or diabetic bladder strips.

3.3. Effect of cromakalim on phasic contractions

Cromakalim had no effect on the amplitude of CCH-induced PA in either control or diabetic bladder strips (Figures 4 & 5). However, cromakalim did significantly decrease the frequency of CCH-induced PA in control tissues at 1μM, 3μM and 10μM and in the diabetic tissues at 3μM and 10μM (Figures 4 & 5).

When expressed as percentage change in the frequency of CCH-stimulated PA in the presence of 3μM and 10μM cromakalim relative to that in the absence of cromakalim (i.e. in presence of 0.5μM CCH only), the percentage inhibition of the frequency of PA at 3μM and 10μM cromakalim was significantly ($p<0.05$) in control tissues (3μM: 34.70±10.41%, 10μM: 41.34±10.08%) than the diabetic tissues (3μM: 11.69±6.13%, 10μM: 18.60±5.70%).

3.3. Effect of K channel blockers on phasic contractions

In order to test the hypothesis that blockade of BK_{Ca} channels would induce PA in bladder strips under resting tension, tissues from control and 1-week diabetic rats were incubated with increasing concentrations (0.01, 0.03 and 0.1μM) of IBTX (Figure 6). IBTX was able to induce PA in tissues from both control and 1-week diabetic animals at the highest concentration (0.1 μM) tested (Figure 6). The effect of 0.1μM IBTX on amplitude of basal PA was significantly ($p<0.05$) greater in 1-week diabetic bladder strips (0.045±0.010g/mg tissue, n=8) than in the control tissues (0.021±0.002g/mg tissue, n=7). There was also no significant difference in the effect of 0.1μM IBTX on the frequency of PA between 1-week diabetic and control tissues.

The effect of the K_{ATP} channel blocker glibenclamide was also investigated in bladder strips from 1-week diabetic rats and their age-matched controls, and is shown in Figure 7.

Glibenclamide failed to induce PA in bladder strips from control and diabetic rats at all concentrations used (Figure 7).

Similarly, the effect of the SK_{Ca} channel blocker apamin was investigated in control and 1-week diabetic rat bladder strips. Typical chart recordings of the effect of apamin are demonstrated in Figure 8. Apamin failed to induce PA in bladder strips from both control and diabetic rats (Figure 8).

4. Discussion

Clinical problems affecting urine storage are commonly associated with detrusor overactivity (DO), which is the presence of inappropriate spontaneous detrusor contractions while the bladder is being filled (Abrams et al, 2002; Drake, 2007). Understanding of the pathophysiological basis of DO and abnormal spontaneous contractions remains incomplete, and the potential for clinical treatment is limited as a result.

Leakage of small amounts of ACH from intramural nerves has been linked to enhanced phasic contractions in overactive bladders during the filling phase of the micturition cycle (Ng et al, 2006), and studies have demonstrated increased phasic bladder contractions upon stimulation with the muscarinic receptor agonist CCH in neonatal rats (Szell et al, 2003). In the present study, in order to mimic this effect and promote the generation phasic contractions, bladder strips were stimulated with a low concentration of CCH. Muscarinic receptor stimulation unmasked significantly higher amplitude of PA in diabetic tissues compared to controls. Similar high amplitude spontaneous contractions have been associated with DO in neonatal rats (Ng et al, 2006). The mechanisms underlying the enhanced CCH-induced PA are unclear, but changes in intracellular Ca^{2+} levels due to alterations in muscarinic receptor function or properties of VDCCs may be important underlying factors. Given the prominent role for K channels in modulating bladder excitability and contractility, we investigated whether a change in the regulation of PA by K channels may explain the enhanced PA in diabetic rat bladder.

Previously an elevated expression of BK channel regulatory α- and β- subunits has been associated with enhanced phasic contractions in the bladder of the STZ-diabetic rat (Nakahara et al, 2004). Thus, in the present study the role of BK channels in modulation of CCH-induced PA was examined. Opening of BK channels via NS1619 had only a minimal effect on PA in both diabetic and control rat bladder strips a significant inhibitory effect observed only at the highest concentration (30μM). This inhibitory effect was abolished in the presence of IBTX (data not shown), indicating a specific action of NS1619 as a BK channel opener. The frequency of PA was unaffected by NS1619, and in addition there was no significant difference in the effect of NS1619 on CCH-induced PA between control and diabetic tissues.

Since NS1619 decreased only the amplitude of PA in bladder strips in the current study, this may demonstrate that this drug is interfering with Ca^{2+} entry and release mechanisms in UBSM. Sheldon *et al* (1997) investigated the effect of NS1619 on detrusor contractility of guinea-pig bladder and demonstrated that NS1619 acts by increasing the open probability of BK channels. Further support comes from Layne et al (2010) who demonstrated that a novel BK channel opener, NS11021, can increase the open probability of BK channels in guinea-pig bladder smooth muscle cells. Opening of BK channels decreased the inward Ca^{2+} current, with a subsequent decrease in the amplitude of PA. Thus, it can be concluded that NS1619 inhibits the amplitude of PA in detrusor smooth muscle cells of the rat bladder by activation of BK channels and inhibition of Ca^{2+} channels, as demonstrated in guinea-pig detrusor (Sheldon *et al*, 1997).

The reason that NS1619 did not inhibit frequency of CCH-induced PA in bladder strips in the present study is not clear, since opening of BK_{Ca} channels is known to be important in restoring the membrane potential and mediating action potential frequency in this tissue. In a recent study by Layne et al (2010), NS11021 significantly decreased the frequency of action potentials in guinea pig UBSM cells, but no decrease in frequency of spontaneous contractions was shown. Indeed changes in UBSM cell membrane potential may not always result in altered UBSM cell contractility. One possibility for the lack of effect of NS1619 on the frequency of PA in the present study could be muscarinic receptor-mediated inhibition of BK channels. Ca^{2+}-activated K currents are inhibited by muscarinic agonists in bladder smooth muscle (Kume and Koltikoff, 1991; Nakamura et al, 2002). Since we stimulated the tissues with a low concentration of carbachol in the present study, it is possible that CCH-induced muscarinic receptor activation may inhibit BK channels in the rat detrusor and thus render the tissue relatively insensitive to the actions of NS1619.

In contrast to the minor effect of the BK channel opener NS1619, the BK channel blocker IBTX had a significant effect on PA. Initiation of PA after administration of IBTX may indicate that in rat UBSM BK channels may be important in the regulation of PA under basal conditions. BK channel blockade has been shown to increase PA in bladder smooth muscle (Imai *et al*, 2001; Buckner

et al, 2002; Darblade *et al*, 2006; Kita et al, 2010). Kita et al (2010) demonstrated an enhanced effect of IBTX in detrusor smooth muscle from obstructed rat bladder with DO compared to sham-operated animals. Only one study, by Nakahara *et al* (2004), has investigated the effect of IBTX in bladders from diabetic rats (8-10 weeks), with IBTX producing a greater effect in diabetic tissues than controls. In the present study IBTX, at the highest concentration (0.1μM), evoked PA of a magnitude similar to that induced by CCH. The IBTX-induced PA was significantly greater in amplitude in diabetic bladder strips compared to controls, whilst the frequency of PA was similar.

A decrease in expression and/or activity of BK channels in the diabetic rat bladder may explain the increased IBTX-induced PA, since a decrease or absence of BK channel function has previously been associated with DO in animal models of bladder dysfunction, such as bladder outlet obstructed rats (Li et al, 2008). In addition, the lack of a feedback pathway from BK channels in UBSM, seen in BK knock out mice (Meredith *et al*, 2004; Thorneloe *et al*, 2005), results in altered excitability of UBSM, detrusor instability and enhanced PA. In our lab reduced levels of BK channel α and β subunits were demonstrated at the mRNA level (unpublished data) and need confirmation at the protein level.

SK channels have an important contributory role in UBSM excitability and contractility. The role of SK channels in modulating the PA has been investigated in studies of mouse and rat bladder (Herrera et al, 2003; Hougard et al, 2009; Kita et al, 2010). These studies have demonstrated that modulation of SK channels is important in mediating phasic contractions of the bladder under pathological conditions, leading to DO in these species. Currently, to our knowledge, there has been only one study investigating the role of SK channels in the modulation of PA in bladder of STZ-diabetic rats (Nakahara *et al*, 2004). In this study, SK channel blockade by the apamin significantly increased PA of detrusor strips from STZ-diabetic rats compared to controls (Nakahara *et al*, 2004). In the present study bladder strips from control and diabetic rats were insensitive to modulation by apamin. This suggests that SK channels may not be important in mediating UBSM cell membrane potential or bladder PA in this species under the current experimental conditions.

K_{ATP} channels in bladder smooth muscle play a critical role in controlling myogenic tone and excitability.. Small increases in K_{ATP} channel activity are likely to move the resting membrane potential away from the threshold of action potential activation and thus have significant inhibitory effects on related PA (Petkov *et al*, 2001). Due to this, K_{ATP} channels have been extensively explored as possible therapeutic targets for treatment of DO (Pinna *et al*, 2005; Elzayat *et al*, 2006; Shieh *et al*, 2007b; Kamiyama *et al*, 2008). The effect of the K_{ATP} channel opener cromakalim on PA has been investigated previously in the guinea-pig and pig bladder (Foster *et al*, 1989; Imai *et al*, 2001; Akino et al, 2008). In these studies, cromakalim significantly inhibited both the amplitude and the frequency of PA. However, in the present study, in contrast to the minimal effect of BK channel opening, which affected only the amplitude of PA, activation of K_{ATP} channels by cromakalim had a significant effect only on the frequency of PA. This suggests that opening of K_{ATP} channels may have a profound effect on action potential activation and firing in UBSM of the rat which underlie the frequency of PA.

Diabetic bladder strips were slightly less responsive to the inhibitory effect of cromakalim compared to controls. This has not previously been reported in the literature, and the reason underlying the difference is not clear. One explanation may be a down-regulation of K_{ATP} channel expression in diabetic bladder resulting in insufficient K_{ATP} channels to produce a functional response to cromakalim. Unpublished preliminary data from our lab has demonstrated a decrease in the mRNA levels of Kir6.1 and SUR2B subunits of KATP channels in 1-week diabetic bladders, but this needs confirmation at the protein level. Muscarinic receptor activation could suppress 40-70% of K_{ATP} currents in UBSM (Bonev and Nelson, 1993b) and trachea (Nuttle and Farley, 1997) through protein kinase C-mediated pathways and these pathways may be enhanced in the diabetic tissue. In contrast to the effect of cromakalim, the K_{ATP} channel blocker glibenclamide failed to induce PA in both control and diabetic rat bladder tissues in this study. A similar finding was observed in human (Darblade *et al*, 2006) and and guinea-pig (Imai *et al*, 2001) UBSM. The lack of response to glibenclamide may indicate that in rat UBSM K_{ATP} channels are closed and thus do not participate in the regulation of PA under basal conditions. 1555

5. Conclusion

In conclusion opening of BK channels produced similar inhibitory effects on CCH-induced PA control and diabetic rat bladder strips, whilst diabetic bladder strips appeared to be less sensitive to the KATP channel openercompared to control bladders. Blockade of BK channels, but not K_{ATP} or SK channels, induced significantly greater PA in bladder strips from diabetic animals compared to controls. This suggests altered BK channel function in the diabetic rat bladder, which may contribute to bladder dysfunction in this model. A better understanding of the K channel defects in the UBSM in this model could be achieved by electrophysiological techniques that allow the study of ion channels function at a single cell level.

Figure 1) Typical chart recordings of the effect of 0.5µM CCH (arrows) on bladder strips from control (a) and 1-week diabetic (b) rats. g = absolute grams of developed tension

Figure 2) Effect of increasing concentrations of NS1619 on the amplitude and the frequency of PA induced by 0.5µM CCH in bladder strips from 1-week diabetic (D) rats and age-matched controls (C). C: n=5 & 1-week D: n=10. **p<0.01 versus paired 0µM NS1619 response. Data is presented as mean±SEM**.** g = absolute grams of developed tension

Figure 3) Effect of increasing concentrations of NS1619 on the amplitude and the frequency of PA induced by 0.5µM CCH in bladder strips from 1-week diabetic (D) rats and age-matched controls (C)**.** C: n=5 & 1-week D: n=10. **p<0.01 versus paired 0µM NS1619 response. Data is presented as mean±SEM.

Figure 4) Typical chart recordings of the effect of increasing concentrations of cromakalim on CCH-induced PA in bladder strips from 1-week diabetic (D) rats and age-matched controls (C). g = absolute grams of developed tension

Figure 5) Effect of increasing concentrations of cromakalim on the amplitude and the frequency of PA induced by 0.5µM CCH in bladder strips from 1-week diabetic (D) rats and age-matched controls (C)**.** C: n=11 & 1-week D: n=14. *p<0.05 & **p<0.01 versus paired 0.5µM CCH response. Data is presented as mean±SEM.

Figure 6) Typical chart recordings of the effect of increasing concentrations of IBTX in bladder strips from 1-week diabetic (D) rats and age-matched controls (C). g = absolute grams of developed tension

Figure 7) Typical chart recordings showing effects of increasing concentrations of glibenclamide on bladder strips from 1-week diabetic rats (D) and age-matched controls (C)**.** g = absolute grams of developed tension

Figure 8) Typical chart recordings of the effect of increasing concentrations of apamin on bladder strips from 1-week diabetic rats (D) and age-matched controls (C).

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