Modulation of ureteric Ca signaling and contractility in humans and rats by uropathogenic *E. coli*

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Floyd RV, Winstanley C, Bakran A, Wray S, Burdyga TV. Modulation of ureteric Ca signaling and contractility in humans and rats by uropathogenic *E. coli*. Am J Physiol Renal Physiol 298:F900–F908, 2010. First published February 3, 2010; doi:10.1152/ajprenal.00468.2009.—Ascending urinary tract infections, a significant cause of kidney damage, are predominantly caused by uropathogenic *Escherichia coli* (UPEC). However, the role and mechanism of changes in ureteric function during infection are poorly understood. We therefore investigated the effects of UPEC on Ca signaling and contractions in rat (*n* = 17) and human (*n* = 6) ureters. Ca transients and force were measured and effects of UPEC on the urothelium were monitored in live tissues. In both species, luminal exposure of ureters to UPEC strains J96 and 536 caused significant time-dependent decreases in phasic and high K depolarization-induced contractions, associated with decreases in the amplitude and duration of the Ca transients. These changes were significant after 3–5 h and irreversible over the next 5 h. The infection causes increased activity of K channels, causing inhibition of voltage-gated Ca entry, and K channel blockers could reverse the effects of UPEC on ureteric function. A smaller direct effect on Ca entry also occurs. Nonpathogenic *E. coli* (TG2) or abluminal application of UPEC did not produce changes in Ca signaling or contractility. UPEC exposure also caused significant impairment of urothelial barrier function; luminal application of the Ca channel blocker nifedipine caused a reduction in contractions as it entered the tissue, an effect not observed in untreated ureters. Thus, UPEC impairs ureteric contractility in a Ca-dependent manner, largely caused by stimulation of potassium channels and this mechanism is dependent on host-urothelial interaction.

Throughout the world urinary tract infection (UTI) is one of the most common infectious diseases seen in general practice, for example accounting for up to 3% of all general practitioner consultations in the United Kingdom (10). Although the majority of these infections remain contained within the bladder, a significant proportion will progress to a more serious infection of the kidneys which can result in renal damage and subsequent renal failure. It is known that ascending UTIs can severely impair contractility of the ureters leading to urinary stasis, which in turn can potentiate the process of infection (15, 39). Most UTIs are caused by uropathogenic *Escherichia coli* (UPEC), with some studies suggesting that 80–95% of all community-acquired infections arise as a consequence of UPEC colonization (14, 17). UTIs therefore remain a consistent and important clinical and economic problem. Despite the existence of several well-characterized animal models of infection, there is a distinct lack of understanding of the events initiated following host-pathogen interaction in the upper urinary tract that cause aberrant functioning of the ureters. Recent work by Lane et al. (24) established that ascending UTI in a mouse model progresses from the bladder to ureters via flagellum-mediated motility, allowing the UPEC to colonize areas of the upper urinary tract including the kidneys. However, the subsequent modulation of normal ureteric smooth muscle function is poorly understood.

Control of normal ureteric contractility in the rat has been well-studied and it has been shown that activity is primarily controlled by Ca entry through L-type voltage-dependent calcium channels following initiation of propagating action potentials (5). Ureteric cell membrane excitability is also regulated by calcium-activated K channels (KCa) and Cl channels (8, 33, 35). The human ureter has been less well-studied but we recently characterized Ca signaling and ion channel expression (13). However, as mentioned above, there are little or no data concerning how these mechanisms are affected by UPEC.

In this study, we report the results of real-time monitoring of ureteric smooth muscle contractility during the process of UPEC infection and investigate possible mechanisms by which impairment of ureteric contractility occurs. We report how the use of a live tissue model, studied using physiological measurements of force and calcium with confocal imaging, allowed us to generate a timeline of the onset of ureteric dysfunction induced by UPEC and to make significant progress in understanding how UPEC interferes with normal ureteric contractility. Using these methods we developed a means of studying the pathophysiology of UPEC-mediated infections in intact preparations.

**METHODS**

**Tissue**. Ureters were removed from adult rats following humane killing and placed in physiological saline before dissection. All experimentation was conducted in accord with the UK Animals (Scientific Procedures) Act 1986. All protocols were approved by the host institution and Local Research Ethics Committee and were designed and performed in accordance with local and national regulations governing research involving humans and animals. The ureters were cut into 1-cm segments and loaded with cell-permeant fluorescent Ca-sensitive indicators indo-1 AM or fluo-4 AM by 3-h incubation in 15 μM indicator with Pluronic F-127 (Molecular Probes/Invitrogen). Following being loaded, tissues were washed in physiological saline and injected with 10^6 CFU/ml *E. coli* before the tissue ends were secured in aluminium foil clips. The middle third segments of six human (4 female, 2 male, age 28–56 yr) macroscopically healthy ureters from live kidney donors for transplantation, with informed consent and local ethical approval, were studied. Tissues were dissected into 1-cm long segments and injected luminally with
10^6 CFU/ml *E. coli* resuspended in physiological saline. The open ends of the ureters were tied off using nonabsorbable surgical suture and tissues were rinsed in saline for 30 min before use.

**Bacterial strains.** J96 and 536 are human pyelonephritis *E. coli* isolates that demonstrate both mannose-sensitive hemagglutination (MSHA) of guinea pig erythrocytes and mannose-resistant hemagglutination of human erythrocytes (1, 18). TG2 is a nonpathogenic strain of *K12 E. coli* that shows MSHA of guinea pig erythrocytes. Hemagglutination activity was studied using previously described methods (19).

**Solutions.** Tissues were superfused at 7–8 ml/min and 30°C (unless stated otherwise) with buffered physiological saline (pH 7.4) with composition (in mM) including 154 NaCl, 5.6 KCl, 1.2 MgSO_4_, 2 CaCl_2_, 8 glucose, and 10.9 HEPES. Calcium-free solutions contained 2 mM EGTA. Contractility was manipulated using 10 μM nifedipine, 5 mM TEA, and 120 mM KCl solution with composition (in mM) including 39.6 NaCl, 120 KCl, 1.2 MgSO_4_, 2 CaCl_2_, 8 glucose, and 10.9 HEPES. Nifedipine was dissolved in ethanol to a final concentration of 0.1% vol/vol, a concentration shown not to have any additional modulatory effects. In some studies, 25 mM methyl α-D-mannopyranoside was added as a competitive inhibitor of type 1 pilus-mediated interactions.

**Confocal microscopy.** Confocal microscopy was performed using an UltraVIEW LCI scanner of the Nipkow disc type, linked to a fast digital camera and UltraVIEW LCI software, attached to Olympus inverted microscope with image acquisition from a large area of the sample at a relatively high speed (20–50 frames/s).

**Ca transients and force.** Indo-1-loaded ureteric preparations were mounted on two stainless steel hooks, one of which was attached to a force transducer and stretched with 25–30% of active force produced by electrical stimulation as spontaneous activity was rarely seen in these preparations (rectangular pulses of 5–7/V and 100-ms duration at 40-s intervals).

**Live staining.** Ureter preparations were incubated in 10 μg/ml wheat germ agglutinin (WGA) conjugated to green fluorescent Oregon Green 488 diluted in physiological saline for 1 h at room temperature. Tissues were washed in fresh physiological saline for 15 min and nuclei were visualized by staining with 1 μg/ml DAPI for 30 min.
min at room temperature. Tissues were washed for a further 15 min before exposure to *E. coli* strains J96 or TG2 and subsequent imaging using confocal microscopy.

**Statistics.** All results are expressed as means ± SE and data were analyzed for statistical difference with Student’s *t*-test with significance taken when *P* < 0.05. Unless stated otherwise, *n* represents observations on different donor ureters or animals. Control samples exposed lumenable to physiological saline showed no significant difference to *E. coli* TG2-exposed samples in all experiments, therefore *E. coli* TG2 data are shown for ease of interpretation.

**RESULTS**

**Effects of UPEC on ureteric contractility.** Phasic contractions of rat ureter were evoked by electrical field stimulation (EFS). Figure 1 shows typical (of 16 others) force records (A) and mean time course data (B) of the effects of luminal applied nonpathogenic (TG2) and pathogenic (J96 and 536) strains of *E. coli* (all at 10⁶ CFU/ml). In contrast to the nonpathogenic strain TG2, the UPEC strains J96 and 536 both produced large time-dependent inhibition of the phasic contractions, reducing their amplitude after 5 h of exposure to 11.2 and 13.4% of control (100%, *P* < 0.01), respectively (Fig. 1B). Methyl α-D-mannopyranoside was able to block the inhibitory effects of J96 and 536 (Fig. 1).

EFS of human ureteric preparations also produced phasic ureteric activity. Similar effects of the UPEC strains on the amplitude of the phasic contractions were also found for human ureter (*n* = 6; Fig. 2A), but with a slower time course for the effects; after 6 h of exposure to UPEC J96, the amplitude of phasic contractions was reduced to 40.1% of control (*P* < 0.01; Fig. 2B).

Following being washed to remove unadhered UPEC from the lumen of the ureters following 5-h exposure and perfusion with physiological saline, no reversal of the fall in force was seen, and indeed a continued decrease was observed during the subsequent 3–5 h (*n* = 5 rat; *n* = 2 human; Fig. 3).

In paired experiments, the effects of luminal and abluminal application of UPEC J96 were compared. Abluminal application of J96 had no effects on ureteric contractility (*n* = 5 rat; 6.1% for TG2 and 6.8% for abluminal J96; Fig. 4B).

**Effects of UPEC J96 on the Ca-force relationship.** The duration and amplitude of phasic contractions in ureteric muscle are largely controlled by the duration of plateau-type action Fig. 4A), and the small decrease in force over the course of 5 h was not significantly different from that observed with nonpathogenic *E. coli* TG2 (6.1% for TG2 and 6.8% for abluminal J96; Fig. 4B).

**Fig. 3.** Irreversibility of the effects of UPEC on rat ureter contractility. Changes in the amplitude of force recorded in intact preparations of rat ureter during luminal exposure to 10⁶ CFU/ml UPEC J96 and following luminal washing with physiological saline.

**Fig. 4.** Role of the urothelium in modulating rat ureter contractility. A: representative traces of force recorded during continuous luminal or external exposure to 10⁶ CFU/ml UPEC J96 for 5 h. B: changes in the amplitude of force recorded in intact preparations of rat ureter during luminal (dotted line) or external exposure (continuous line) to 10⁶ CFU/ml UPEC J96 where 10-min periods of activity from each sample at each time point were compared and used to generate B.
potentials, which open L-type Ca channels and activate Ca influx. This global rise of Ca activates the contractile response (7). In addition, Ca-independent modulation of force can occur via the RhoA/Rho-kinase pathway (5, 33). The following set of experiments was designed to investigate the effect of UPEC on the force-Ca relationship in the rat ureter. As can be seen from Fig. 5, A and B, the decrease in the amplitude and duration of the phasic contractions induced by UPEC J96 was closely correlated with the decrease in the amplitude and duration of the Ca transient. The amplitude and duration of both force and calcium transients were found to decrease in a time-dependent manner (see Fig. 5A and inset). In these experiments, 5-h exposure of Indo-1-loaded ureteric strips to UPEC J96 produced a decrease in the amplitude of phasic contraction and Ca transient of 13.4 ± 0.6% (P < 0.05) and 50.0 ± 0.6% (P < 0.05), respectively. The durations of force and Ca transient measured at 50% of peak (t50) were from 2.97 ± 0.35 to 1.51 ± 0.13 s (P < 0.01) and from 2.5 ± 0.3 to 1.21 ± 0.15 (P < 0.01), respectively (n = 14; Fig. 5B). There was no significant effect of UPEC on the force-Ca relationship during the rising or the relaxation phase of the phasic contractions, suggesting that UPEC did not modulate force in a Ca-independent manner (Fig. 5C).

Effects of UPEC in the presence of K channel blocker. The amplitude and duration of the Ca transient and force in ureter muscle are tightly coupled to the parameters of the action potential (4, 6). Thus, one could suggest that inhibition of the Ca transient induced by UPEC could be caused by either inhibition of L-type Ca channels or activation of K channels, or a combination of the two. To test the possible role of K channels in the observed inhibition of the Ca transient, ureteric segments were luminaly exposed to UPEC J96 or 536 for 5 h and every hour for 10 min, the effects of tetraethylammonium (5 mM TEA, a K channel blocker) on Ca transients and force were investigated. As shown in Fig. 6Ai, TEA produced the anticipated elevation in Ca signals and contractility under control conditions. Figure 6Aii shows, in the same preparation, the large decrease in Ca and force after 5-h exposure to J96. Then, as shown, TEA application significantly restored the amplitude of force, from 13.4 to 75% of control amplitude. The mean data for J96 and 536 are presented in Fig. 6B and are significantly different compared with control values from 3 h onwards (P < 0.05, n = 14). The calcium transient also showed significant restoration in amplitude and duration (Fig. 6A).

To study the effects of UPEC on calcium influx via L-type Ca channels, the effects of nonpathogenic and pathogenic strains of E. coli on Ca transients and force induced by high-K+ depolarization were investigated. High-K+ depolarization induced a contractile response, consisting of an initial phasic component followed by a more or less sustained tonic component associated with a similarly biphasic Ca transient (Fig. 7A). Superimposed records of typical force (top traces) and associated Ca transients (bottom traces) recorded in control conditions and after 5-h exposure to nonpathogenic (TG2) and pathogenic (J96) strains of E. coli are shown in Fig. 7Ai and ii, respectively (n = 6). These records show that nonpathogenic E. coli TG2 had little or no effects on both high K-induced force and Ca transients (Fig. 7Ai). In contrast, UPEC J96 produced a decrease in the amplitude of both the phasic and sustained component of force and the Ca transient (Fig. 7Aii).

Fig. 5. Mechanism of UPEC action on rat ureter contractility. A: representative traces of simultaneous recordings of force and calcium [Ca], (Indo-1, ratio of fluorescence at 400:500 nm) recorded during continuous luminal exposure to E. coli and in inset, superimposed single transients taken at 0 (i), 3 (ii), and 5 (iii) h of exposure. B: changes in the duration of force (black bar) and calcium transients (gray bar) at 50% of peak exposure to 10⁶ CFU/ml UPEC J96. C: superimposed records of the force/Ca relationship recorded during the rising and relaxation phases of the normalized phasic contractions and ratios in control conditions (black) and after 3-h exposure to UPEC J96 (green) taken from A. *Significant reduction compared with controls P < 0.05.
Within 5 h of inoculation with E. coli J96, significant numbers were assessed pharmacologically using luminal application of facet cell exfoliation. Conversely, exposure to TG2 caused insignificant levels of ureteric preparations.

The observation that urothelial cells show increased expression of inducible nitric oxide (NO) synthase in response to bacterial invasion leading to an elevation of NO, a known bacterial product, our observations are consistent with the idea that UPEC evoked a sustained response to impair ureteric contractility or that there may be urothelium-dependent irreversible changes which cause upregulation of K channels. Although washing may not be 100% effective at removing bacteria or bacterial products, our observations are consistent with the idea that UPEC J96 binds to ureteric urothelial cells, initiating complex signaling cascades that may impair ureteric activity. As suggested by others (22), the active secretion of bacterial products may play a key role in this effect given that no significant inhibitory effect was caused by either heat-killed E. coli or bacterial lysates (data not shown). This interpretation is further supported by the relatively slow onset of inhibition which occurred over a period of hours, as opposed to a previous report of acute blockade of activity in less than 10 min (22). The slower onset of inactivation of contractility that we found is consistent with observations in previous studies that demonstrated invasion of urothelial cells occurring 1–3 h after inoculation (21), which initiates modifications in signaling and transcription involving RhoA, Cdc42, and Rac1 (26). Furthermore, studies of mouse bladder showed that during this early phase of infection, the superficial facet cell layer undergoes rapid turnover and regeneration in an effort to clear the internalized bacteria (29, 30). Similar evidence for facet cell exfoliation was found in our studies of human and rat ureters 5 h postinoculation with UPEC J96.

The observation that urothelial cells show increased expression of inducible nitric oxide (NO) synthase in response to bacterial invasion leading to an elevation of NO, a known

The functional effects on urothelial barrier permeability were assessed pharmacologically using luminal application of 10 μM nifedipine, which blocks calcium entry through L-type calcium channels. When applied abuminally to reach the ureteric myocytes, phasic contractions were abolished within 3–5 min (n = 5 rat; Fig. 9A). Luminal exposure of control rat ureters to nifedipine caused no significant decrement of force even after 5 h, suggesting that the urothelial barrier is impermeable to this drug under control conditions (Fig. 9B). Following 5 h of luminal exposure to UPEC J96, which inhibits contractility to just 13.4% of control values, UPEC suspensions were replaced with 10 μM nifedipine (n = 5). This resulted in an immediate and further significant depression of contraction by 52 ± 0.46% (P < 0.05) within 5 min (Fig. 9C), suggesting that the tight barrier function has been compromised by exposure to UPEC. No significant decrease in contractility was seen following TG2 exposure and subsequent application of nifedipine.

DISCUSSION

These data provide convincing evidence to suggest that UPEC impairs ureteric contractility via a calcium-dependent mechanism that is dependent on host-urothelium interaction. To the best of our knowledge, these are the first studies to demonstrate the real-time process of infection and its effects on contractility and calcium signaling in live intact ureteric preparations.

Infection with UPEC strains J96 and 536 significantly depressed normal phasic activity in rat and human ureters within 2 and 3 h, respectively, by a mechanism involving activation of K channels and inhibition of calcium entry through L-type calcium channels. Further analysis of ureters exposed to UPEC J96 but washed with normal physiological saline and observed over a 5-h recovery period suggests that either the adhered UPEC evoked a sustained response to impair ureteric contractility or that there may be urothelium-dependent irreversible changes which cause upregulation of K channels. Although washing may not be 100% effective at removing bacteria or bacterial products, our observations are consistent with the idea that UPEC J96 binds to ureteric urothelial cells, initiating complex signaling cascades that may impair ureteric activity.

Figure 7B shows mean data for the effects of the two E. coli strains on the sustained component of force and Ca transient expressed as a percentage of the peak. The amplitude of the sustained component of force and Ca transient was decreased by 35 ± 0.53 and 29 ± 2.9% (P < 0.05), respectively, after 5 h of exposure to UPEC J96.

UPEC J96 causes urothelial facet cell loss and breakdown of the urothelial barrier. Urothelial barrier function is a critical factor in maintaining protection of ureteric contractility (28). Live staining of infected human and rat ureters was performed to label cell boundaries (green) with Oregon-Green 488 WGA and nuclei (blue) with DAPI (Fig. 8). In both human and rat ureters, the large facet cells that line the ureter can clearly be seen under control conditions (Fig. 8, A and D, respectively). Within 5 h of inoculation with UPEC J96, significant numbers of facet cells had been exfoliated 68 ± 0.45% (P < 0.05) to reveal the smaller, tightly packed underlying urothelial cells in both human (Fig. 8B) and rat (Fig. 8E) ureteric preparations. Conversely, exposure to TG2 caused insignificant levels of facet cell exfoliation 4 ± 0.02%.

The functional effects on urothelial barrier permeability were assessed pharmacologically using luminal application of

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**Figure 6.** Role of K channels in UPEC action of rat ureter contractility. A: representative traces of force calcium (Ca) (Indo-1, ratio of fluorescence at 400:500 nm) recorded during control (i) and continuous luminal exposure to E. coli J96 for 5 h (ii) and response to 5 mM TEA under both conditions, i.e., control (black) and 5 h (red). B: changes in the amplitude of force recorded in intact preparations of rat ureter during luminal exposure to 10⁶ CFU/ml UPEC J96 or 536 (dotted lines) and response to 5 mM TEA for 10 min every hour (continuous lines) where 10-min periods of activity from each sample at each time point were compared and used to generate B. *Significant restoration of activity compared with controls P < 0.05.
smooth muscle relaxant, may contribute to the effects on ureteric contractility (32). The urothelium of proximal rat ureter is known to produce NO but it is not normally found in the smooth muscle cells (27). Given that our data suggest that UPEC J96 causes a breakdown in the integrity of the urothelial barrier, as demonstrated by the effect of nifedipine following inoculation with the bacteria, there is a significant risk of urothelium-derived factors gaining access to the smooth muscle layer.

Urothelial integrity plays a key role in regulating the contractility of the underlying ureteric smooth muscle in rats, as removal of the urothelial cell layer initiates spontaneous contractile activity and modified agonist-induced responses (28). Furthermore, there is good evidence that the urothelium of both bladder and ureter secretes an as yet unidentified inhibitory factor which prevents aberrant contractility of the underlying smooth muscle (9, 16, 28, 40). There is increasing evidence that UPEC can modulate calcium signaling in urothelial cells through several mediators. Altered urothelial calcium signaling can modulate gene transcription (12), stimulate cytokine release (38) mediated by LPS and TLR4, and can be initiated by the interaction of UPEC adhesin FimH with the integral membrane protein uroplakin IIIa to cause caspase-dependent urothelial apoptosis in the bladder (23, 41). Secreted α-hemolysin can initiate formation of transient cation-selective transmembrane pores in the host cell that cause modified calcium signaling and initiate potassium efflux (2, 11, 42) without increasing the ability of the invading strain to colonize its host (34, 37). These previous observations are consistent with the contractile phenotype observed in rat ureters exposed to UPEC J96 and 536, pyelonephritis strains that both contain two copies of the α-hemolysin operon hlyCABD (3, 31). Given that Hly has been shown to cause extensive damage to murine bladder urothelium in the early stages of infection (37) and initiates exfoliation of differentiated human bladder urothelial cells (36), the observed decrease in urothelial cell number in response to UPEC challenge in the present study is consistent with Hly playing a key role in modulating urothelial responses to infection. As secretion of Hly can be correlated clinically with increased severity of human UTI (20, 25), these data suggest a significant functional role for Hly in evoking urothelial damage and modulating cell signaling which may initiate the contractile changes observed in this study.

Although availability of human tissue is limited, our data suggest that its responses, both physiologically and to infection, are similar to those of rat ureter, which should therefore be a useful model for further future mechanistic studies. This is consistent with our earlier findings concerning the characteristics of Ca signaling and contractility in human ureter (13).

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Fig. 7. Role of calcium channels in UPEC action on rat ureter contractility. A: representative trace of 120 mM KCl-induced force (top) and calcium transient (bottom) recorded in intact preparations of rat ureter during luminal exposure to 10^6 CFU/ml nonpathogenic E. coli TG2 (i) or UPEC J96 (ii) at 0 (black) and 5 h (red). B: effects of E. coli strains TG2 and J96 on the sustained component of force (left) and Ca transients (right) expressed as a percentage of the peak of both recorded in intact preparations of rat ureter following 5-h luminal exposure to 10^6 CFU/ml UPEC J96 or nonpathogenic E. coli TG2. *Significant reduction compared with controls and TG2 P < 0.05.
Although our sample number is limited in size, the responses to UPEC were consistent and seen in all tissues.

The effects on contractility initiated by UPEC J96 are mannose sensitive (Fig. 1.), suggesting a role for type 1 fimbriae (19). However, E. coli TG2, although expressing type 1 pili, does not cause similar effects on contractility. This suggests that although type 1 pili may be required for adherence, other bacterial factors also contribute to the observed changes in ureteric contractility.

In conclusion, we showed that UPEC causes significant inhibition of contractility in intact rat and human ureteric preparations via a urothelial-dependent interaction that initiates inhibition of L-type calcium channels and activation of K channels in smooth muscle cells. As yet, the mechanisms mediating these effects are unknown and clearly warrant further study.

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**DISCLOSURES**

No conflicts of interest are declared by the authors.

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