MAJOR ARTICLE

Escherichia coli–Mediated Impairment of Ureteric Contractility Is Uropathogenic *E. coli* Specific

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(See the editorial commentary by Nicolle, on pages 1494–6.)

Background. Ureters are fundamental for keeping kidneys free from uropathogenic *Escherichia coli* (UPEC), but we have shown that 2 strains (J96 and 536) can subvert this role and reduce ureteric contractility. To determine whether this is (1) a widespread feature of UPEC, (2) exhibited only by UPEC, and (3) dependent upon type 1 fimbriae, we analyzed strains representing epidemiologically important multilocus sequence types ST131, ST73, and ST95 and non-UPEC *E. coli*.

Methods. Contractility and calcium transients in intact rat ureters were compared between strains. Mannose and *fim* mutants were used to investigate the role of type 1 fimbriae.

Results. Non-UPEC had no significant effect on contractility, with a mean decrease after 8 hours of 8.8%, compared with 8.8% in controls. UPEC effects on contractility were strain specific, with decreases from 9.47% to 96.7%. Mannose inhibited the effects of the most potent strains (CFT073 and UTI89) but had variable effects among other UPEC strains. Mutation and complementation studies showed that the effects of the UTI89 cystitis isolate were *fimH* dependent.

Conclusions. We find that (1) non-UPEC do not affect ureteric contractility, (2) impairment of contractility is a common feature of UPEC, and (3) the mechanism varies between strains, but for the most potent UPEC type 1 fimbriae are involved.

Urinary tract infections (UTIs) are the most common laboratory-confirmed infections in Europe and North America, accounting for substantial medical costs worldwide [1]. Most UTIs are caused by uropathogenic *Escherichia coli* (UPEC). Indeed studies suggest that 80%–95% of all community-acquired infections arise as a consequence of UPEC colonization [1, 2]. As the ureters perform an essential function in the transportation of urine from the kidneys to the bladder, efficient ureteric contractility and peristalsis is vital in preventing renal infection. Peristalsis is brought about by propagating action potentials giving rise to global Ca transients, which underlie waves of phasic contractions of the smooth muscle that pass down the ureteric walls [3]. It has been shown that *E. coli* colonization can impair ureteric peristalsis, which in turn potentiates the infection due to urinary stasis and/or vesicoureteric reflux [4, 5]. A better understanding of the potential impact of uropathogenic *E. coli* on ureteric peristalsis may help us to understand the pathogenesis of renal infection.

E. coli are generally classified according to their clinical properties and carriage of virulence genes, which broadly classifies pathovars as either diarrheagenic or

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extraintestinal. Extraintestinal *E. coli* such as UPEC possess virulence characteristics that allow them to persist in and colonize regions outside the gastrointestinal tract.

Many factors have been implicated in the virulence of UPEC infections, including adhesins (type 1, S/F1C, and P pili [fimbriae]) [6–8], hemolysin, autotransporter toxin, siderophores, capsule, and cytotoxic necrotizing factor 1 [9, 10].

UPEC encode numerous adhesive pilus fibers assembled by the chaperone/usher pathway [11] that are often tipped with adhesins that bind to receptors with stereochemical specificity [12], thus facilitating colonization and biofilm formation [13]. All UPEC encode the class of chaperone/usher pathway pili known as type 1 pili that are tipped with the mannose-binding FimH adhesin. FimH is known to bind mannosylated proteins such as uroplakins that coat the luminal surface of both the human and murine bladder [14]. FimH has been shown to facilitate bacterial colonization and invasion of human bladder cells [15]. Internalized UPEC can escape into the host cell cytoplasm, where they are able to subvert expulsion and innate defenses by aggregating into biofilm-like intracellular bacterial communities in a FimH-dependent process [13]. Intracellular bacterial community formation has been documented in exfoliated bladder epithelial cells in the urine of a small proportion of women presenting with acute uncomplicated urinary tract infection [16]. In agreement with these findings and in support of a role for FimH in humans, it has been shown that the *fimH* gene is under positive selection in human clinical isolates of UPEC [7].

Whole-genome sequencing has been used to identify UPEC-associated pathogenicity islands [17–19]. There have also been a number of studies reporting the distribution of specific genomic regions among UPEC isolates [20–23], indicating that not all *E. coli* isolates associated with UTI carry UPEC-associated virulence genes. Various studies have demonstrated the presence of virulence genes normally associated with UPEC in diarrheagenic *E. coli* [24] and avian pathogenic *E. coli* (APEC) [21, 25], suggesting that other non-UPEC strains might have the potential to act as uropathogens. The majority of drug-resistant UPEC causing UTI in the United Kingdom are members of an epidemic clone of the ST131 lineage, first reported in January 2008 [26, 27]. Isolates of the ST131 clone are significant contributors to UTIs and are often associated with invasive disease and/or high-level antimicrobial resistance [27–31]. ST131 isolates have also been recovered from the intestines of healthy humans [32]. Other lineages of epidemiological importance are ST73 and ST95 [31].

We have previously characterized the morphology, Ca signals, and contractility patterns in animal [33] and human [3] ureter tissues and have validated the rat as a good experimental model for the human ureter and for the study of UPEC infection [3, 34]. In addition, we have shown that 2 strains of UPEC isolated from human pyelonephritis patients (J96 and 536) have an inhibitory effect on ureteric function in both species, which is thought to be mediated by type 1 fimbriae [34].

The aim of this study was to analyze epidemiologically important UPEC and a selection of non-UPEC strains in our well-characterized live tissue model, to allow real-time monitoring of ureteric smooth muscle contractility and calcium signaling during exposure to bacteria. We report that the inhibitory effect is restricted to UPEC but shows variation between strains. In addition, we report further analysis of the role of type 1 fimbriae in the effect of UPEC on ureteric contractility.

MATERIALS AND METHODS

Bacterial Strains Used in This Study

A total of 6 UPEC, 1 APEC, 2 enteropathogenic *E. coli* (EPEC), and 1 chicken gut commensal strain (Table 1) were tested along with a relevant nonpathogenic TG2 (K-12 derivative) strain and saline as controls. Two examples of ST131 strains from a previous survey in northwest England [31] were tested (EC958 and M160), along with 2 examples of ST73 strains (M9 and M12). Strain UTI89 (ST95) [19] and CFT073 (ST73) [17] were included as well-characterized genome-sequenced UPEC strains also representing common multilocus sequence types. Two EPEC strains, E2348/69 [35] and D55 [36], and 1 APEC strain, 3770 (isolated from the reproductive tract of a chicken), along with a chicken commensal strain, 5138 (isolated from chicken gut), were also included. To investigate the role of type 1 fimbriae in mediating the observed changes in ureteric function, several *fim* mutants were studied. These included a full *fim* operon deletion mutant SJH-1106 (UTI89 Δ*fim*) (Chen and Hultgren, unpublished data), a *fimH* deletion mutant SLC2-17-fimH (UTI89 Δ*fimH*) [7], and a *fimH* deletion complemented with wild-type *fimH* SLC2-33-1(UTI89 Δ*fimH*/p*fim*H) [7].

Preparation of Bacterial Suspensions

Suspensions of *E. coli* were prepared using methods established for use in a murine model of UTI [37]. Briefly, *E. coli* were streaked onto Luria agar plates to obtain single colonies, which were then used to inoculate 10 mL of Luria broth in 125-mL Erlenmeyer flasks. Cultures were incubated statically overnight at 37°C to ensure optimal type 1 pilus expression. The following day, a 25-μL volume of each strain was subcultured into 25 mL of Luria broth in a 250-mL Erlenmeyer flask and incubated overnight, as before. Following static serial passage, cultures were pelleted at 5000 *g* for 5 minutes at 4°C. The pellet was resuspended in sterile physiological saline or, for type 1 pilus inhibition studies, in sterile physiological saline containing 25 mM methyl α-D-mannopyranoside (MαDM), and was diluted until the OD_{600} for each strain corresponded to $1–2 \times 10^7$ colony-forming units (CFU) per 50 μL.

Table 1. Collated Data Showing the Percentage Inhibition of Contractile Amplitude After Exposure to Each Strain of *Escherichia coli* for 8 Hours, Compared With Saline Control

Strain	Pathotype	Reference/Source	No.	Inhibition, % (−MαDM)	P[a]	Inhibition, % (+MαDM)	P[b]	Agglutination Titer[c]
EC958 (ST131)	UPEC	*E. coli* clinical isolate; UK [43]	7	78.03	< .0005	82.06	NS	1:64
M160 (ST131)	UPEC	*E. coli* clinical isolate; Upton (unpublished)	11	75.98	< .0005	11.67	< .0005	1:128
M9 (ST73)	UPEC	*E. coli* clinical isolate; Upton (unpublished)	5	42.18	< .0005	38.33	NS	1:128
M12 (ST73)	UPEC	*E. coli* clinical isolate; Upton (unpublished)	5	9.47	NS	9.75	NS	1:64
UTI89 (ST95)	UPEC	Cystitis *E. coli* isolate [19]	6	96.75	< .0005	10.24	< .0005	1:1024
CFT073 (ST73)	UPEC	Pyelonephritis *E. coli* isolate [17]	5	87.93	< .0005	9.45	< .005	1:512
2348/69	EPEC	Enteropathogenic *E. coli*; gift from J. N. Fletcher [35]	5	9.82	NS	…	…	1:16
D55	EPEC	Enteropathogenic *E. coli*; gift from J. N. Fletcher [36]	5	8.7	NS	…	…	1:16
3770	APEC	Chicken isolate; gift from P. Wigley	5	8.41	NS	…	…	1:512
5138	None	Chicken commensal strain; gift from P. Wigley	5	8.39	NS	…	…	1:32
TG2	None	K-12 derivative; laboratory control strain	7	8.67	NS	…	…	1:64
Control	NA	Physiological saline	8	8.77	…	…	…	…
UTI89 Δ*fimH*	UPEC	Knockout of *fimH* in UTI89 SLC2-17-fimH [7]	5	10.24	NS	…	…	ND
UTI89 Δ*pfimH*	UPEC	Knockout of *fimH* in UTI89 complemented with wt *fimH* SLC2-33-1 [7]	5	43.92	< .0005	…	…	ND
UTI89 Δ*fim*	UPEC	Full *fim* operon deletion SJH-1106; Chen and Hultgren unpublished	5	9.09	NS	…	…	ND

Strains causing significant inhibition were also assessed in the presence of 25 mM methyl α-D-mannopyranoside (MαDM). Data are the results of 4 independent experiments.

Abbreviations: NA, not applicable; ND, not done; NS, not significant; UPEC, uropathogenic *E. coli*; +, present; –, absent.

[a] Compared with saline control at 8 h. A P value of > .05, by an unpaired t test, is statistically significant.

[b] Compared with −MαDM at 8 h. A P value of > .05, by an unpaired t test, is statistically significant.

[c] Agglutination titers of *Saccharomyces cerevisiae* in the presence and absence of 3% w/v MαDM are shown for each strain of *E. coli*.

Ureteric Contractility Experiments

Adult female rats (Charles River, United Kingdom) were killed humanely by use of CO_2 anesthesia followed by cervical dislocation in accordance with United Kingdom legislation, and ureters were removed and placed in physiological saline before dissection. Ureters were cut into 1-cm lengths and loaded with 15 μM of cell permeant fluorescent Ca-sensitive indicator indo-1 AM in Pluronic F-127 (Molecular Probes/Invitrogen, Paisley, United Kingdom) for 3.25 hours. Loaded tissues were washed in physiological saline and injected with $2–4 \times 10^6$ CFU in physiological saline, using a 33G needle and disposable sterile syringe before securing the tissue ends in aluminum foil clips.

Tissues were superfused at 7–8 mL/min and 30°C (unless stated otherwise) with buffered physiological saline (pH 7.4) composed of 154 mM NaCl, 5.6 mM KCl, 1.2 mM $MgSO_4$, 2 mM $CaCl_2$, 8 mM glucose, and 10.9 mM HEPES. In some studies, 25 mM MαDM was added as a competitive inhibitor of type 1 pilus–mediated interactions.

For measurements of intracellular calcium, Indo-1 was used as described elsewhere [3, 34]. Loaded ureteric preparations were mounted on 2 stainless steel hooks, one of which was attached to a force transducer, and stretched (passive force being 25%–30% of the active force produced by electrical field stimulation to set optimal length, as spontaneous activity was rarely seen in these preparations). Rectangular pulses of 5–7 V and 100 ms duration were applied at 40-second intervals.

Agglutination of *Saccharomyces cerevisiae*

E. coli strains were assessed for their ability to aggregate yeast cells to assess the expression of functional type 1 pili [38].

Yeast agglutination assays were performed as described by Li et al [39]. Briefly, static overnight cultures of *E. coli* were washed by repeated centrifugation in phosphate-buffered saline and resuspended to an OD_{600} of 1. Commercial baker's yeast (*S. cerevisiae*) suspensions (1% w/v) containing 0.01% (w/v) Brilliant Blue R-250 were prepared in sterile phosphate-buffered saline with or without 3% (w/v) MαDM. Serial dilutions of bacterial suspensions were made in V-bottomed 96-well plates (1:2) and mixed with an equal volume of yeast suspension. After 1 hour at room temperature, the agglutination titer was determined as the last dilution yielding positive aggregations. Unless otherwise stated, all stock salts and buffers were purchased from Sigma-Aldrich (Dorset, United Kingdom).

Statistics

A sample size of 5 animals per strain was calculated using GraphPad StatMate with estimates SD based on previously published data from similar control and treatment groups in the same animal model [34]. All results were expressed as means ± standard error (SE), and data were analyzed for statistical difference with an unpaired *t* test, with significance taken when $P < .05$. Unless stated otherwise, "n" represents observations on different animals.

RESULTS

Effects of *E. coli* Strains in the Intact Rat Ureter Model

Phasic contractions were evoked in rat ureters by electrical field stimulation, and changes in phasic activity and calcium transients were studied over time following inoculation with 4×10^6 CFU *E. coli*. Figure 1 shows representative mean data that demonstrate the time-dependent decrease in amplitude of phasic contractions that occurred over time with *E. coli* strains exhibiting high (CFT073 [ST73]), intermediate (M9 [ST73]), and low (3770 [APEC]) inhibitory activity, when compared to initial amplitude (taken for 100%).

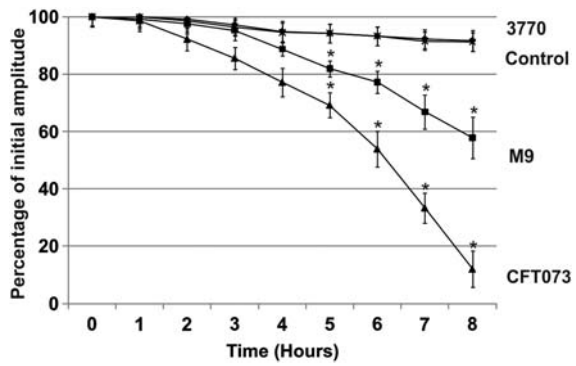

Figure 1. Effects of *Escherichia coli* applied to rat ureteric lumen. Example traces of strains causing low (3770), intermediate (M9), and high (CFT073) impairment of ureteric contractile amplitude over time. Statistical significance was determined by an unpaired *t* test ($P < .0005$) is denoted by an asterisk.

Data summarizing the effect of all *E. coli* strains tested on ureteric contractility are given in Table 1. Both ST131 UPEC strains EC958 and M160 produced significant time-dependent inhibition of phasic contractions, reducing their amplitude after 8 hours of exposure to 22% ± 1.6% and 24% ± 9.7% of control, respectively. No significant difference in contractile amplitude was observed after 8 hours in ureters inoculated with nonpathogenic TG2 (91.3% ± 3.2% of initial amplitude), compared with those containing physiological saline alone (91.2% ± 3.2% of initial amplitude).

Figure 1 shows mean data recorded from ureters exposed lumenally to ST73 isolate M9, which caused a 42.2% ± 7.3% fall in the amplitude of phasic contractions after 8 hours of exposure (Table 1). However, no significant deterioration of ureteric contractility was seen in samples exposed to ST73 strain M12, (90.5% ± 2.3% of initial amplitude), when compared to TG2 and physiological saline.

Of all the UPEC isolates tested, CFT073 (ST73) and UTI89 (ST95) produced the most aggressive response, restricting ureteric activity to small, nonpropagating contractions after 8 hours of exposure (12.1% ± 6.3% and 3.3% ± 4.7% of initial amplitude for CFT073 [ST73] and UTI89 [ST95], respectively). Figure 1 shows the typical functional deterioration observed in ureters exposed to CFT073 (ST73) over time.

Typical traces of phasic activity and calcium transients recorded during exposure to CFT073 (ST73), demonstrated that the loss of activity is mediated by depression of the duration and amplitude of the calcium transient that drives contractility (Figure 2*C*). This effect is consistent with our previous studies investigating the effect of well-characterized pyelonephritis strains of *E. coli* J96 and 536 in intact rat ureters [34], which showed similar mean inhibition (88.8% and 86.6% of control, respectively).

Prolonged exposure of rat ureters to the EPEC strains 2348/69 or D55 did not cause any significant impairment of ureteric contractility over an extended period of 12 hours, when compared to physiological saline (9.8% ± 0.6%) or TG2 (8.7% ± 0.4%) nonpathogenic controls, respectively (Table 1). Mean collated data at each time point shows that although there was a small decrease in amplitude over time, this did not differ significantly from control values at the same time point (data not shown).

Rat ureters exposed to strains 3770 and 5138 showed no significant impairment of contractile function over the course of an 8-hour experiment. Representative force and calcium traces from exposure to 3770 did not show any marked decrease of activity over time (Figure 2*A*). These strains resulted in only 8.4% ± 0.7% and 8.4% ± 0.3% reductions in amplitude, respectively, over the duration of exposure (Table 1). These data are comparable with the normal average decreases

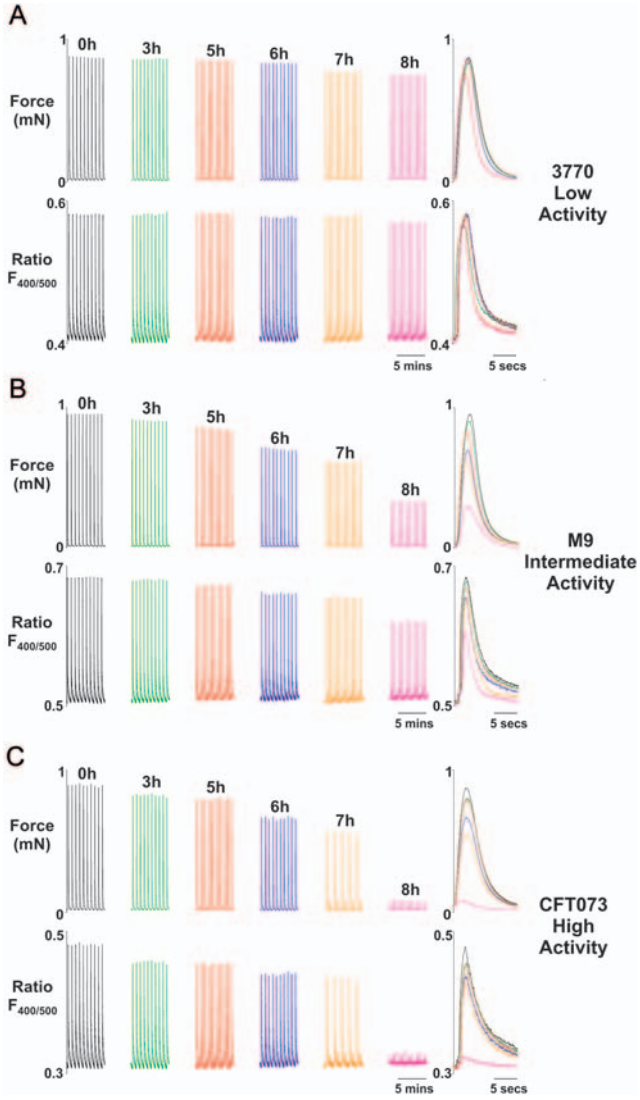

Figure 2. Effects of *Escherichia coli* applied to rat ureteric lumen on force and calcium transients. Example recordings of force and calcium transients from rat ureters loaded with calcium-sensitive Indo 1 and exposed to *E. coli* strains that exhibit low (A, 3770), intermediate (B, M9), and high (C, CFT073) impairment of ureteric contractile amplitude over time. Figures show that impaired ureter activity is mediated by a decrease in the duration and amplitude of the calcium transient.

observed in control samples. Together, these data show that neither EPEC nor APEC or chicken commensal strains caused impairment of function or calcium signaling in an intact tissue model of ureteric infection over 8 hours.

Effect of Mannose on UPEC Inhibition of Contractility

A dose of 25mM MαDM was able to block the inhibitory effects of M160 (ST131; 11.7% ± 1.3%) (Figure 3) but not EC958 (ST131; 82.1% ± 1.6%) (Table 1). In ureters exposed to M9 (ST73), MαDM was unable to completely block the inhibitory effects of M9 on contractile amplitude but the onset of ureteric impairment was delayed by 2 hours (42.2% ± 7.3 for –MαDM, compared with 38.3% ± 3.5 for +MαDM). The inhibitory effects of UPEC strains UTI89 (ST95) and CFT073 (ST73), which caused the largest impairment of contractility, were abolished in the presence of 25 mM MαDM (10.2% ± 1.7% and 9.5% ± 2.3%, respectively).

Type 1 Fimbrial Mutants of UTI89 (ST95) Have Reduced Ability to Inhibit Contractility

Deletion of the UTI89 (ST95) *fim* operon eradicates its ability to impair ureteric contractility. Rat ureters exposed to the full *fim* operon deletion mutant (UTI89 Δ*fim*) showed only small, statistically insignificant decreases in contractile amplitude after 8 hours of exposure (9.1% ± 0.2%) (Figure 4), when compared to the wild-type parental UTI89 (ST95) strain, which induced a 96.8% reduction in contractile amplitude (Table 1). In addition, no significant change in contractile amplitude was observed in ureters exposed lumenally to UTI89 (ST95) lacking *fimH* (10.2% ± 0.8%), suggesting that contractile impairment is mediated in part by FimH-mediated binding of bacteria with host urothelium. The inhibitory capacity was partially restored (43.9% ± 5.2%) when UTI89 Δ*fimH* was complemented with wild-type *fimH* (UTI89 Δ*fimH*/p*fim*H) (Figure 4).

Agglutination of *S. cerevisiae*

The prevalence of type 1 fimbriae was determined by the presence or absence of mannose-sensitive yeast agglutination (MSYA) of *S. cerevisiae* (Table 1). Strains showing the greatest impairment of contractility also demonstrated the highest MSYA titer (1:1024 for UTI89 [ST95] and 1:512 for CFT073 [ST73]). UPEC strains that demonstrated intermediate effects on contractile amplitude showed MSYA titers of 1:128 (M160 and M9). Surprisingly, EC598 showed a comparably low MSYA titer (1:64) while retaining a high capacity for contractile impairment. This level of MSYA was also seen in UPEC M12, a strain that showed no statistically significant attenuation of ureter contractile amplitude. However, APEC 3770 demonstrated MSYA at similar levels to CFT073 (ST73) without inducing any significant change in contractility. MSYA was observed in EPEC strains 2348/69 and D55, as well as in APEC 5138, at titers of 1:16 for EPEC and 1:32 for APEC; none of these strains showed any significant inhibition of ureter function. These data suggest that mannose-binding capacity does not consistently correlate with effects on ureteric function.

DISCUSSION

These data provide the first evidence to suggest that impaired ureteric contractility during infection is a general feature of UPEC but that other *E. coli*, such as EPEC and APEC, do not cause a similar effect. We have also found that the strength of

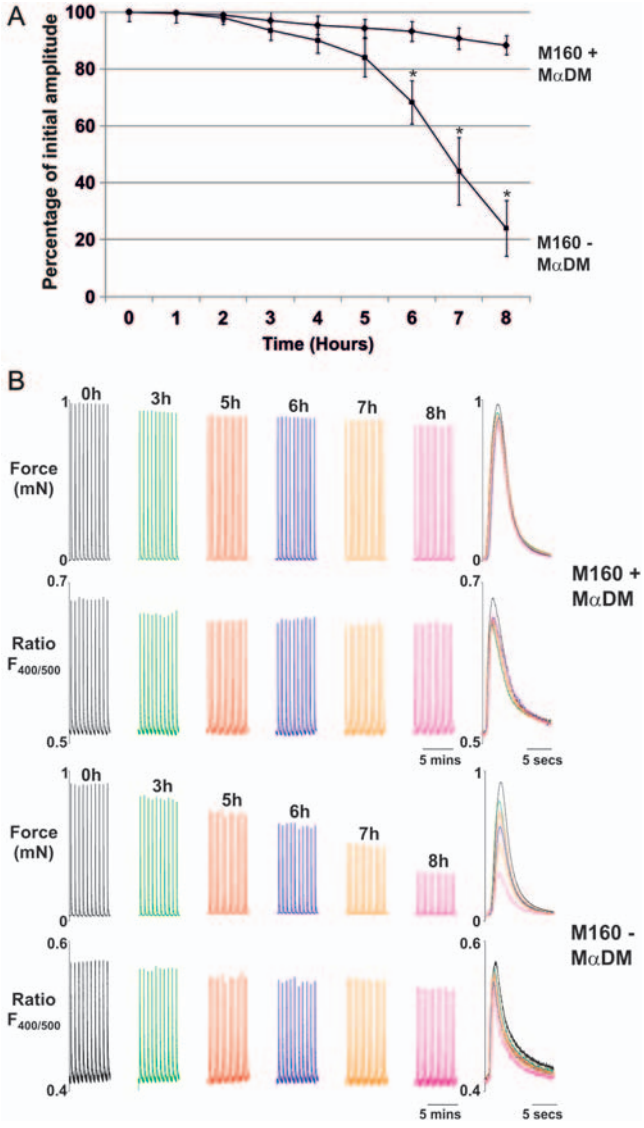

Figure 3. Effects of methyl α-D-mannopyranoside (MαDM) on inhibition of phasic ureteric activity mediated by uropathogenic *Escherichia coli* (UPEC). *A*, Contractile amplitude in rat ureters exposed lumenally to UPEC M160 in the presence and absence of 25 mM MαDM is shown. *B*, Example recordings of force and calcium transients from rat ureters loaded with calcium-sensitive Indo 1 and exposed to M160 in the presence and absence of 25 mM MαDM are shown. Statistical significance was determined by an unpaired *t* test ($P < .0005$) is denoted by an asterisk.

the effect varies between UPEC strains and that there are strain variations with respect to the role of type 1 fimbriae.

One of our UPEC strains (M12 [ST73]) did not exhibit any effect at all. This strain had low agglutination activity, suggesting that production of type 1 fimbriae is impaired. The reasons for this are not known, but it is possible that this strain is carrying a mutation that impacts its production of type 1 fimbriae. Differences in adhesion, even in the presence of equivalent levels of type 1 fimbriation, have previously been reported in populations of *E. coli* isolated from different host niches. Such strains displayed structural differences in the *fimH* gene that can impair D-mannose-sensitive adhesion [40].

The effects on contractility mediated by UTI89 (ST95), CFT073 (ST73), and M160 (ST131) are mannose sensitive, indicating that the initial cascade of events is mediated by type 1 fimbrial binding to mannose residues expressed on urothelial cells [41]. This is supported by our studies using specific type 1 fimbrial deletion mutants of strain UTI89. Small-molecular-weight compounds that specifically inhibit the FimH type 1 pilus lectin can prevent and treat UPEC infections in an in vivo mouse model of UTI [42]. Consequently, these compounds may also reduce the ability of mannose-sensitive UPEC, such as UTI89 (ST95), CFT073 (ST73), and M160 (ST131), to decrease contractility. However, mannose-sensitive agglutination was not found to correlate with functional effects on ureter contractility in all strains tested. Although APEC 3770 produced similar levels of MSYA to CFT073 (ST73), no significant change in contractility was observed. Similar evidence for normal mannose-binding capacity but attenuated in vivo fitness of *E. coli* has previously been demonstrated in UPEC UTI89 (ST95) having an A27V/V163A double mutant in *fimH*. The A27V/V163A mutant strain had no effect on mannose binding in vitro. However, compared with wild-type, this double mutant strain exhibited a 10 000-fold reduction in mouse bladder colonization 24 hours after inoculation and was unable to form intracellular bacterial communities in a mouse model of UTI even though it bound normally to mannosylated receptors in the urothelium [7]. The impaired contractility caused by exposure to EC958 (ST131) was resistant to MαDM inhibition, suggesting that the observed ureteric dysfunction might be mediated by an alternative mechanism involving other chaperone/usher pathway adhesins, such as S, F1C, or P fimbriae. Genome sequence analysis has shown that strain EC958 carries an inactivating insertion in the *fimB* gene, which is consistent with the observed resistance to MαDM [43]. Relatively little is known about the specific virulence mechanisms that make ST131 UPEC suited to causing invasive disease in humans. Studies of virulence marker prevalence in a collection of first nonduplicate isolates (n = 300) recovered from urine specimens in 2 diagnostic laboratories in northwest England found a low prevalence of several UPEC-associated virulence genes [31]. Consequently, although there is significant evidence to suggest that toxins produced by UPEC, such as hemolysin and cytotoxic necrotizing factor 1, might contribute to the observed effects on contractility [10], the absence of *hlyA* and *cnf1* genes in EC958 suggest that the mechanism varies between strains. Other virulence factors, such as autotransporters, which are widely expressed in UPEC, including those in our study [43], and include secreted autotransporter toxin (Sat)

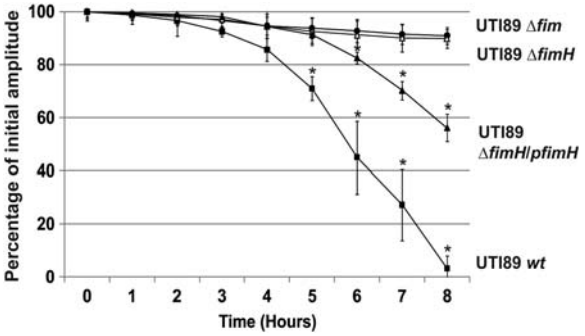

Figure 4. Effect of type 1 fimbrial mutants on phasic activity in rat ureters. The effects of UTI89 Δ*fim,* UTI89 Δ*fimH* and UTI89 Δ*fimH*/p*fim*H on phasic activity in rat ureters over time are shown. Statistical significance was determined by an unpaired *t* test ($P < .0005$) is denoted by an asterisk.

[10], surface-located UpaH [44], and trimeric UpaG [45], may also be important in this response in some strains.

It has been suggested that APEC might serve as a reservoir of plasmid-mediated virulence genes transmissible to other bacteria [21]. Given the lack of physiological evidence in our studies for a role in affecting intact tissue preparations directly, this would seem a more plausible role for APEC in mediating human urinary tract disease rather than acting as a uropathogen themselves. Interestingly, comparative studies of virulence genes in 524 APEC and 200 UPEC isolates identified substantial areas of overlap between the 2 groups: APEC showed a 39.1% frequency of the *papG* allele II, compared with 24% of UPEC [21]. The class II *papG* allele is specifically associated with human pyelonephritis and bacteremia. Conversely, the class III *papG,* which is associated with human cystitis, was only found with a low frequency in APEC (0.6% of isolates), compared with UPEC (25.5% of isolates) [46–48]. The frequency with which *papG* II is detected in APEC, coupled with the almost ubiquitous expression of the *fimH* adhesin of the type 1 pilus (98.1% of isolates), provides evidence that certain subgroups of APEC might harbor the ability to bind to human urothelial cells. We found no evidence that the APEC strain tested in this study had an effect on ureter contractility, but it may be that there are variations among APEC strains.

Infection with UPEC strains UTI89 (ST95), CFT073 (ST73), EC958 (ST131), M160 (ST131), and M9 (ST73) significantly depressed normal phasic activity in rat ureters within 5 hours, with some strains completely abolishing activity after 8 hours. The delayed onset of contractility impairment is consistent with current understanding that the initial phase of infection involves colonization and invasion of urothelial cells 1–3 hours after inoculation [49]. The changes in contractility were found to result from reductions in the intracellular calcium transients that underlie force production, as clearly seen in Figures 2 and 3. Thus, irrespective of the different virulence mechanism, a reduction in calcium signaling is the common final pathway [50]. This study, coupled with our previous studies, demonstrates that this experimental animal model provides a good representation of events occurring in human ureter tissues during the initial stages of infection [34]. Our study indicates that an ability to impact on ureteric contractility is a widespread feature of UPEC but that the mechanisms involved may vary between strains.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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