Uropathogenic *Escherichia coli* Alters Muscle Contractions in Rat Urinary Bladder via a Nitric Oxide Synthase–Related Signaling Pathway

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**Background.** Uropathogenic *Escherichia coli* (UPEC) is a common cause of urinary-tract infection. The mechanisms by which bacteria cause the symptoms of cystitis remain unclear.

**Methods.** The contractions of isolated rat detrusor strips evoked by electrical field stimulations (EFS) or by exogenous agonists and immunoblotting for the detection of protein expression in the bladder were measured in the short (1 h) and long (24 h) term after the intravesical instillation of J96 (O4:K6) strain or UPEC isolated from patients with acute *E. coli* pyelonephritis or *E. coli* lipopolysaccharide (LPS).

**Results.** One hour after the instillation of UPEC, the level of endothelial nitric oxide synthase (eNOS) and the contractile response, but not protein kinase C (PKC) and extracellular signal-regulated kinase (ERK)–1/2 activation, were higher. Twenty-four hours after UPEC treatment, detrusor contractions were decreased, and inducible (i) NOS protein expression and ERK1/2 phosphorylation, but not PKC activation, were observed. Both aminoguanidine and PD98059 treatment markedly reversed the decrease of EFS- and acetylcholine-evoked detrusor contractions induced by UPEC. The instillation of LPS triggered PKC activation but not ERK1/2 phosphorylation.

**Conclusions.** Short-term intravesical instillation of UPEC enhances detrusor contractions through an eNOS-related pathway, but iNOS-regulated ERK1/2 signaling may be involved in long-term UPEC treatment–induced responses. There are different mechanisms involved in the responses induced by UPEC and LPS.

The development of urinary-tract infections, including cystitis and pyelonephritis, in critically ill adult patients is associated with considerable morbidity, prolonged hospitalization, and greater health care expenditures [1]. Urinary-tract infection is also one of the most common bacterial infections in children, ranking second only to those of the respiratory tract [2]. It has been indicated, on the basis of precise genetic techniques, that *Escherichia coli* strains residing in the rectal flora serve as a reservoir for urinary-tract infections (e.g., cystitis) [3]. The most common pathogens of cystitis are similar all over the world: 80%–90% of cases are caused by *E. coli* [4]. However, the exact mechanisms by which bacteria cause the symptoms of cystitis are poorly understood.

Nitric oxide (NO) plays an important physiologic role in the relaxation of the urethral sphincter during micturition, and its predominant site of action is at the level of the urethral smooth-muscle cells [5]. James et al. [6] suggested that a relaxation mechanism in the human isolated detrusor strips in response to electrical field stimulation (EFS) was at least partly mediated via the production of NO. It has been found that intra-vesical NO donors were capable of suppressing bladder hyperactivity induced by cyclophosphamide-induced cystitis [7]. Inducible NO synthase (iNOS) was originally identified in activated murine macrophages and was induced by inflammatory mediators in a number of cell types [5]. A direct relationship existed between nitrite levels in urine and urinary-tract infection [8]. Both uropathogenic *E. coli* (UPEC) [9] and lipopolysaccharide (LPS) [10] have been found to be capable of inducing iNOS expression in the urinary bladder. Moreover, endothelial (e) NOS is expressed primarily.

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in the caveolae of epithelial and endothelial cells [11]. eNOS has also been localized in mouse bladder and ureteral urothelium [12]. Kang et al. [13] found that LPS induces a rapid up-regulation of eNOS, which can be phosphorylated by activated Akt (a signaling protein activated by phosphatidylinositol 3-kinase) in the bladder. However, the role that NOS plays in the regulation of detrusor muscle tone during urinary-tract infection remains unclear. Moreover, we have previously demonstrated that β-phorbol-12,13-dibutyrate (a protein kinase C [PKC] activator) enhanced the contractile responses evoked by EFS in mouse and rat urinary bladder [14, 15]. Extracellular signal-regulated kinase (ERK), a subfamily of mitogen-activated protein kinases (MAPK), has also been shown to play an important role in the regulation of PKC-mediated uterine artery contractility in pregnant sheep [16]. It has also been demonstrated that ERK is involved in regulating angiotensin II– and endothelin-1–induced contractions of rat thoracic aorta [17]. The functional importance of ERK and PKC in the regulation of detrusor muscle tone during urinary-tract infections is also still unclear.

In the present study, we focused on the effects of human pyelonephritogenic E. coli J96 (O4:K6) strain or UPEC isolated from patients with acute E. coli pyelonephritis on rat detrusor muscle contractions and the possible signaling pathway involved in the UPEC-induced responses. We determined the effects of the intravesical instillation of UPEC on NOS protein expression, the activation of ERK1/2 and PKC, and detrusor muscle contractions in rat urinary bladder, compared with the effects of LPS, the endotoxin from E. coli.

**MATERIALS AND METHODS**

**Bacterial strain.** The human pyelonephritogenic E. coli strain J96 serotype O4:K6 (ATCC 700336), which expresses type 1 and P-fimbrial adhesions, was used. The receptor-binding function of type 1 pili present on strain J96 has been identified in establishing experimental rat bladder infections [18]. E. coli isolated from the urinary culture of a human with acute E. coli pyelonephritis (gift from Dr. P. R. Hsueh, Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan) was also used. In addition, LPS prepared by trichloroacetic acid extraction from E. coli serotype 026:B6 was purchased from Sigma.

**Induction of inflammation.** Adult female Wistar rats (weight, 180–230 g) were used. The Animal Research Committee of the College of Medicine, National Taiwan University, Taipei, Taiwan, approved conducted the study in accordance with the guideline for the care and use of laboratory animals. Rats were anesthetized with ketamine (30 mg/kg) and xylazine (4 mg/kg) and then instilled intravesically with UPEC (10⁶ cfu in 500 μL of PBS) or protamine (10 mg in 500 μL of sterile PBS) followed by LPS (1 mg in 500 μL of sterile PBS) [15, 19]. In some experiments, rats were instilled intravesically with N⁵-nitro-l-arginine methyl ester (L-NAME; an NOS inhibitor; 5 mg in 500 μL of sterile PBS) or aminoguanidine (an iNOS inhibitor; 10 mg in 500 μL of sterile PBS) or PD98059 (an ERK-kinase [MEK] inhibitor; 20 μg in 500 μL of sterile PBS) 30 min after the instillation of UPEC or LPS.

**Preparation of detrusor strips.** After each rat was killed, the lower abdomen was opened, and the exposed bladder was excised by a cut above the trigone. The isolated bladder was washed with several changes of modified Krebs solution (130.6 mmol/L NaCl, 4.8 mmol/L KCl, 1.2 mmol/L MgSO₄, 12.5 mmol/L NaHCO₃, 2.5 mmol/L CaCl₂, and 11.1 mmol/L glucose) and opened by 2 lateral incisions to give a rectangular sheet of tissue. Detrusor strips (6 mm long and 2 mm wide) were suspended in a 10-mL organ bath between built-in vertical platinum electrodes. Initial tension was set at 1 g, which was found in preliminary experiments to be the optimal tension for this preparation. Tissues were allowed to equilibrate for 60 min, during which Krebs solution was changed and replaced with fresh solution. The preparation was maintained at 37.0°C ± 0.5°C and oxygenated with 95% O₂ and 5% CO₂. The pH of the Krebs solution was 7.2–7.4. Strips were stimulated with supramaximal trains of pulses (pulse duration, 0.2 ms; train duration, 10 s) at frequencies of 4 and 16 Hz. The tension was recorded through an isometric transducer (Grass FT.03) on a data-acquisition system with analytical software (Biopac Systems). Some isolated detrusor strips were used to obtain contractile responses to exogenous agonists (acetylcholine and α,β-methylene ATP).

**Western-blot analysis.** Tissues were homogenized in buffer that contained 20 mmol/L HEPES, 0.25 mol/L sucrose, 0.5 mmol/L EDTA, 2 mmol/L dithiothreitol, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL leupeptin, 10 μg/mL apro- tinin, and 1 mmol/L sodium orthovanadate (Na₂VO₃) (pH 7.5). The cell homogenates were precleared from nuclei and cell debris by centrifugation at 10,000 g for 15 min at 4°C. The supernatant (total cell lysate) was then ultracentrifuged at 100,000 g for 1 h at 4°C, which resulted in a supernatant, referred to as the "cytosolic fraction" and in a pellet (the crude membrane fraction). For PKC immunoblotting, the pellet was resuspended in 250 μL of homogenizing buffer and 1% (vol/vol) of nonidet P-40 and incubated on ice for 30 min, followed by centrifugation at 100,000 g for 30 min at 4°C. The supernatant fraction was termed the "membrane fraction." Western-blot analysis for the presence of particular proteins or for phosphorylated forms of proteins was performed on total cell lysates; the PKC isoform distribution was performed on cytosolic and membrane fractions. Total protein containing 30–80 μg was separated on 8% SDS-polyacrylamide minigels and transferred to nitrocellulose membranes. After blocking, blots were incubated with antibodies for iNOS, eNOS, PKC isoforms (α and
\( \delta \) (BD Transduction Laboratories), phospho-eNOS, and phospho-PKC (pan; Cell Signaling) in PBS/Tween 20 for 1 h, followed by 2 washes in PBS/Tween 20, and then incubated with horseradish peroxidase–conjugated goat anti–mouse IgG for 30 min. Moreover, \( \alpha \)-tubulin served as a control for sample loading and integrity. The antibody-reactive bands were revealed by the enhanced chemiluminescence kit (Amersham) and were used to expose to Kodak radiographic film. The amount of polypeptide was quantitated by integrated densitometric analysis of the film (Kodak Gel Logic-100 Imaging System). Densitometric readings were corrected for the amount of protein in the samples loaded onto the gels. The distribution of PKC isoforms is presented as the PKC membrane:cytosolic ratio.

Reverse transcription–polymerase chain reaction (RT-PCR) for iNOS expression. The expression of iNOS in bladder was determined by RT-PCR analysis. Tissues were homogenized with 1 mL of Trizol reagent (Gibco), and total RNA was isolated in accordance with the manufacturer’s protocol. First-strand cDNA was synthesized by the extension of (dT) primers with 200 U of SuperScript II reverse transcriptase (Gibco) in a mixture that contained 1 \( \mu \)g of total RNA digested by RNase-free DNase (2 U/\( \mu \)g of RNA) for 15 min at 37\(^{\circ}\)C. The cDNA was then used as a template in a PCR using the Perkin Elmer DNA Thermal Cycler Model 480. PCR was performed in a final volume of 50 \( \mu \)L that contained all 4 dNTPs, 1.5 mmol/L MgCl\(_2\), 2.0 U of AmpliTaq (Gibco), and 0.4 \( \mu \)mol/L each primer. iNOS sense 5’-CCCTTCCGAAGTCTGAGCCAGCAG-3’ and antisense 5’-GGGTCTCTCAGGAGTTGTTGGCCC-3’ allowed amplification of a product at 474 bp. The amplification cycles were 45 s at 94\(^{\circ}\)C, 45 s at 65\(^{\circ}\)C, and 2 min at 72\(^{\circ}\)C. The PCR products were separated by electrophoresis on a 1.8% agarose gel after 30–35 cycles and visualized by ethidium-bromide staining. The mRNA of \( \beta \)-actin served as control for sample loading and integrity.

Measurement of nitrite/nitrate (NOx) levels in urine and bladder tissue. Nitrite was assayed using the NOx assay kit (Assay Designs). The procedures for sample handling and assay were in accordance with the manufacturer’s instructions. All assays were performed in duplicate.

Statistical analysis. Data are expressed as the mean ± SE. Significant differences from the respective control values for each experimental test condition were assessed using analysis of variance and the Bonferroni \( t \) test. \( P < .05 \) was considered to be significant.

RESULTS

eNOS expression, phosphorylation of ERK1/2 and PKC, and contractile responses in rat urinary bladder 1 h after the intravesical instillation of UPEC. One hour after the intravesical instillation of UPEC (J96 or \( E. \) coli isolated from patients with acute \( E. \) coli pyelonephritis), levels of eNOS and phosphorylated eNOS proteins were markedly higher (figure 1A), whereas iNOS was not detected (data not shown). Moreover, UPEC treatment did not affect the phosphorylation of ERK1/2 and PKC (pan) (figure 1B and 1C). In some experiments,
Figure 2. Muscle contractions evoked by electrical field stimulation (EFS) or exogenous acetylcholine (ACh) in the urinary bladder 1 h after uropathogenic Escherichia coli (UPEC) treatment. Detrusor muscle strips isolated from rats 1 h after the intravesical instillation of J96 or E. coli isolated from patients with acute E. coli pyelonephritis (EC) were contracted by EFS (10 s at 4 and 16 Hz) (A) or ACh (B). In some experiments, rats were instilled intravesically with N(G)-nitro-L-arginine methyl ester (L-NAME) 30 min after the instillation of UPEC (C). Data are the mean ± SE (n = 6). *P < .05 vs. the control group. #P < .05 vs. the UPEC group.

Figure 3. Western-blot analysis of inducible nitric oxide synthase (iNOS) protein expression in the urinary bladder and kidney after the intravesical instillation of UPEC. Twenty-four hours after the intravesical instillation of UPEC and LPS, iNOS proteins were detected in the bladder (figures 3 and 4A). UPEC, but not LPS, could also induce iNOS expression in the kidney (figure 3; data for J96-induced iNOS expression not shown). Three hours after UPEC treatment, iNOS mRNA was also detected in rat bladder (figure 4B). Levels of NOx in the urinary bladder of UPEC-treated rats were higher (1-h treatment: J96, 141.6% ± 9.2% of control, and E. coli isolated from patients, 154.2% ± 10.3% of control, n = 4; 24-h treatment: J96, 279.6% ± 18.1% of control, and E. coli isolated from patients, 246.9% ± 16.7% of control; n = 4 each). These UPEC- or LPS-induced responses could be reversed by aminoguanidine.

okadaic acid (1 μmol/L), a serine/threonine phosphatase inhibitor, was added to Western-blot samples (figure 1A, middle, 1B, bottom, and 1C, bottom). Urinary NOx levels were higher in UPEC-treated rats (J96, 156.3% ± 7.4% of control; E. coli isolated from patients, 167.2% ± 8.6% of control; n = 5).

One hour after the intravesical instillation of UPEC, contractile responses of the isolated rat detrusor strips evoked by EFS (4 and 16 Hz) or by the exogenous agonist acetylcholine (3 μmol/L) were significantly higher in the UPEC groups than in control groups (figure 2). L-NAME (a nonspecific NOS inhibitor, in that it can inhibit all NOS isoforms), instilled intravesically 30 min after UPEC instillation, significantly reversed the enhancement of EFS- and acetylcholine-evoked detrusor contractions induced by UPEC (figure 2C). UPEC did not affect the exogenous agonist α,β-methylene ATP–induced contractile responses (data not shown).

iNOS expression, ERK1/2 phosphorylation, and PKC translocation and contractile responses in rat urinary bladder 24 h after the intravesical instillation of UPEC. Twenty-four hours after the intravesical instillation of UPEC and LPS, iNOS proteins were detected in the bladder (figures 3 and 4A). UPEC, but not LPS, could also induce iNOS expression in the kidney (figure 3; data for J96-induced iNOS expression not shown). Three hours after UPEC treatment, iNOS mRNA was also detected in rat bladder (figure 4B). Levels of NOx in the urinary bladder of UPEC-treated rats were higher (1-h treatment: J96, 141.6% ± 9.2% of control, and E. coli isolated from patients, 154.2% ± 10.3% of control, n = 4; 24-h treatment: J96, 279.6% ± 18.1% of control, and E. coli isolated from patients, 246.9% ± 16.7% of control; n = 4 each). These UPEC- or LPS-induced responses could be reversed by aminoguanidine.
Figure 4. Expression of inducible nitric oxide synthase (iNOS) in the urinary bladder after the intravesical instillation of uropathogenic Escherichia coli (UPEC). Urinary bladders were isolated from rats 3 or 24 h after the intravesical instillation of J96 or E. coli isolated from patients with acute E. coli pyelonephritis (EC). In some experiments, rats were instilled intravesically with aminoguanidine (AG) or PD98059 (PD) 30 min after the instillation of UPEC. A, Representative typical Western blots of iNOS protein in the bladder isolated from rats 24 h after UPEC treatment. α-tubulin served as a control (C) for sample loading and integrity. B, top, Typical gel electrophoresis of reverse transcription–polymerase chain reaction of iNOS mRNA in bladders isolated from rats 3 h after UPEC treatment. The mRNA of β-actin served as a control for sample loading and integrity. Results shown are representative of at least 4 experiments.

Figure 5. Effects of uropathogenic Escherichia coli (UPEC) or lipopolysaccharide (LPS) on the stimulation of extracellular signal-regulated kinase (ERK)–1/2 mitogen-activated protein kinase (MAPK) in the urinary bladder. Urinary bladders were isolated from rats 24 h after the intravesical instillation of E. coli isolated from patients with acute E. coli pyelonephritis (EC) or with J96 or LPS in the presence or absence of aminoguanidine (AG) or PD98059 (PD). For measurement of the ERK1/2 phosphorylation and protein expression, Western-blot analysis was performed using anti-phospho (p)–ERK1/2 MAPK and anti-ERK1/2 MAPK antibodies. A, Representative typical Western blots of ERK1/2 in bladder. B, Analysis of the relative amounts of pERK1 in bladders treated with UPEC, relative to control. Data are mean ± SE (n = 5). *P < .05 vs. control group. #P < .05 vs. UPEC groups.

(a selective iNOS inhibitor) [20] (figures 3 and 4). However, PD98059 (an ERK/MAPK inhibitor) did not change the effect of UPEC on the induction of iNOS expression (figure 4A).

ERK1/2 phosphorylation in the bladder was markedly induced by treatment with UPEC for 24 h but not by treatment with LPS (figure 5). Total ERK1/2 protein levels were not affected. Moreover, aminoguanidine and PD98059 treatment significantly attenuated ERK1/2 phosphorylation (figure 5).

The distribution of PKCα and PKCδ between the cytosolic and membrane fractions was analyzed by Western blotting. PKCα proteins were markedly reduced in the membrane fractions of LPS-treated group, a phenomenon that resulted from the translocation of the enzymes to the membrane during their activation, but there was no such reduction in the UPEC-treated group (figure 6). Aminoguanidine significantly reversed the inhibition of PKCα distribution by LPS. Neither LPS nor UPEC affected the distribution of PKCδ between the cytosolic and membrane fractions in the bladder (figure 6).

Contractile responses of the isolated rat detrusor strips evoked by EFS (4 and 16 Hz) or by acetylcholine were significantly decreased in the UPEC groups, compared with the control group, when measured 24 h after the intravesical instillation of UPEC (figure 7). UPEC did not affect the contractile responses induced by α,β-methylene ATP (data not shown). Moreover, both aminoguanidine and PD98059 markedly reversed the decrease of EFS- and acetylcholine-evoked detrusor...
contractions induced by UPEC (figure 7). Aminoguanidine or PD98059 alone did not affect the detrusor contractile responses (data not shown).

DISCUSSION

The intravesical instillation of LPS, the endotoxin of E. coli, into rat bladders after pretreatment with protamine sulfate has been shown to cause a localized inflammatory response similar to that seen in urinary-tract infections [10, 19]. The instillation of protamine sulfate followed by the instillation of LPS for 1 h significantly induced rat detrusor hyperreflexia [21]. The exogenous application of the bacterial exotoxin N-formyl-methionyl-leucyl-phenylalanine has also been shown to cause a significantly large, monophasic contracture in rabbit detrusor during a 10-min period of toxin exposure [22]. Nevertheless, it has been demonstrated that the contractility of the rat urinary bladder was decreased during sepsis induced by cecal ligation and perforation [23]. Previously, we also found that, 24 h after the intravesical instillation of LPS, the contractile responses of the isolated rat detrusor strips evoked by EFS are significantly lower [15]. However, little is known about the effect of UPEC on the contractility of the urinary bladder. The present study has shown that contractions of the isolated rat detrusor strips evoked by EFS or the exogenous agonist acetylcholine were enhanced at 1 h and decreased at 24 h after the intravesical instillation of UPEC in rats. However, LPS instilled intravesically did not affect exogenous agonist acetylcholine–evoked de-
trusor contractions in rat bladders [15]. These results imply that there are different mechanisms involved in the alteration of detrusor contractions induced by UPEC and LPS.

Urine during urinary-tract infections has been found to contain an isoform of NOS, an endogenous source of nitrite in urine [24]. The urinary nitrite levels of wild-type mice, but not of iNOS-deficient mice, increased after infection with UPEC [25]. It has been demonstrated that UPEC induces iNOS expression in the mouse bladder and kidney [9]. iNOS expression has also been found to be induced in the rat urinary bladder [10, 15], but not in the kidney [10], after the intravesical instillation of LPS. Recently, Kang et al. [13] demonstrated that eNOS played a role in the early response to bladder inflammation with LPS. They further demonstrated that Akt and eNOS are both localized in the bladder urothelium and that the phosphorylation of eNOS by Akt provides an attractive mechanism for rapid increases in urinary NO production [13].

Higher eNOS expression has also been observed in bladder-biopsy samples from patients with neurogenic detrusor overactivity [26]. In the present study, 1 h after UPEC treatment, levels of eNOS and phosphorylated eNOS protein were markedly higher (figure 1), whereas iNOS was not detected (data not shown), and levels of urinary NOx in UPEC-treated rats were also higher. The NOS inhibitor L-NAME could inhibit the UPEC-induced enhancement of detrusor contractions. On the other hand, we also found that UPEC treatment induced a significant increase in mRNA message for iNOS in rat bladders. iNOS proteins were also induced in rat bladders 24 h after the intravesical instillation of UPEC, whereas eNOS phosphorylation was not detected (data not shown). The iNOS inhibitor aminoguanidine could inhibit the UPEC-induced inhibition of detrusor contractions. The changes in NOS in rat bladder induced by UPEC treatment are similar to the effects of LPS reported in previous studies [10, 13, 15]. These findings imply that an eNOS-related pathway participates in the early contractile response and that the iNOS-related pathway is involved in the late response to UPEC-triggered bladder inflammation. Moreover, it has been shown that iNOS is notably distinguished from the constitutive isoforms by its prolonged production of a relatively large amount of NO [5]. We therefore infer that NOx levels in the bladder may be an important factor.

We then measured the NOx levels in rat bladders, and the results showed that NOx levels in the urinary bladder after 24 h of treatment of E. coli were higher than those after 1 h of treatment. Nevertheless, the real mechanism should be subjected to further investigation.

The proliferation of normal uroepithelial cells has been shown to be associated with the activation of MAPK pathways [27]. MEK/ERK activity has been demonstrated to be involved in the down-regulation of the pressure-dependent myogenic tone of the uterine artery during pregnancy [28]. It has also been shown that MEK-ERK inhibition partially reverses cardiac contractile failure caused by endothelin-1 and isoprenaline co-stimulation [29]. Chen et al. [30] indicated that apoptosis in renal tubular cells induced by UPEC toxins was dependent on ERK1/2. Chen et al. [31] further demonstrated that renal tubular cells are susceptible to E. coli toxins, which induce iNOS-mediated cell death—a process that is regulated by ERK. In the present study, results revealed that the phosphorylation of ERK1/2 in the bladder was significantly induced 24 h after the instillation of UPEC but not after only 1 h. Aminoguanidine, an iNOS inhibitor, was capable of inhibiting UPEC-induced ERK1/2 phosphorylation. These results imply that an iNOS-related ERK signaling pathway is involved in the long-term, but not the short-term, response to UPEC-induced bladder inflammation. However, the intravesical instillation of LPS did not affect the phosphorylation of ERK1/2. Our study’s next aim was to ascertain whether the inhibition of iNOS and ERK would affect the contractile response of detrusor muscle under long-term UPEC treatment. For studying this issue, aminoguanidine or PD98059 (an MEK inhibitor) was used. Aminoguanidine and PD98059 markedly reversed the inhibitory effects of UPEC on the detrusor contractions evoked by EFS or exogenous acetylcholine. These findings indicate that an iNOS-regulated ERK1/2 signaling participated in causing the inhibition of detrusor contractions induced by UPEC bladder instillation.

The detrusor smooth muscle is the main muscle component of the urinary bladder wall. Contractile activation of the detrusor smooth muscle is initiated by the release of transmitters from motor nerves [32]. The facilitative presynaptic muscarinic mechanism that markedly enhances acetylcholine release during continuous EFS has been demonstrated to be dependent on a PKC-mediated second messenger pathway and on the influx of extracellular Ca2+ into the parasympathetic nerve terminals via L- and N-type Ca2+ channels [33]. A recent study showed that pregnancy selectively enhanced the role of ERK in α1-adrenoceptor-mediated contractions and its effect in suppressing the PKC-mediated contraction in the uterine artery [16]. Muscarinic receptors mediate normal bladder contraction as well as at least the main part of contraction in the overactive bladder. In the present study, we found that the contractions of the isolated detrusor strips evoked by EFS were altered after the intravesical instillation of UPEC, similar to the effects of LPS [15]. Moreover, the acetylcholine-evoked detrusor contractions could also be increased at 1 h but decreased at 24 h after the intravesical instillation of UPEC. However, LPS did not affect the acetylcholine-evoked detrusor contraction [15]. These results indicate that changes in the contractile responses of the urinary bladder by UPEC are caused by possible alterations in muscarinic receptor characteristics. By contrast, LPS may have neurogenic effects on the contractile responses of the
urinary bladder [15]. Moreover, the role that PKC plays in the alteration of contractile responses in the bladder during urinary-tract infection remains unclear. For studying this issue, we measured PKC (pan) phosphorylation and the distribution of PKCa and PKCα between the cytosolic and particulate fractions in the bladder. Unexpectedly, the results showed that LPS, but not UPEC, impaired the translocation of PKCa from the cytosolic fraction to the particulate fraction and that this was blocked by treatment with aminoguanidine. Neither LPS nor UPEC affected the translocation of PKCα in the bladder. Therefore, unlike LPS, the PKC-related pathway seems to not be involved in the UPEC-induced alteration of detrusor contraction. However, the murine bladder is innervated mainly by cholinergic (acetylcholine) and nonadrenergic noncholinergic (purinergic or ATP) mechanisms [34]. UPEC (data not shown) and LPS [15] did not affect the contractile responses induced by exogenous agonist α,β-methylene ATP, which indicates that UPEC and LPS did not affect the postsynaptic purinergic receptor-effector pathways. However, the causes of the different mechanisms in bladder responses induced by UPEC and LPS are still in need of further clarification.

In conclusion, UPEC was capable of enhancing urinary bladder muscle contractions through an eNOS-related pathway during short-term intravesical instillation. Nevertheless, an iNOS-regulated ERK1/2 signaling pathway may participate in causing the inhibition of urinary bladder muscle contractions induced by long-term UPEC treatment. There are different mechanisms involved in the responses induced by UPEC and LPS.

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References


