Rapid Up-Regulation of Endothelial Nitric-Oxide Synthase in a Mouse Model of Escherichia coli Lipopolysaccharide-Induced Bladder Inflammation

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ABSTRACT

Increases in the signaling molecule nitric oxide (NO) during inflammation may be linked not only to inducible nitric-oxide synthase (iNOS) but also to endothelial (e)NOS. Escherichia coli lipopolysaccharide (LPS) induces an inflammatory response in the bladder and rapidly increases phosphorylation of Akt/protein kinase B (Akt), a key enzyme regulating proliferation, apoptosis, and inflammation. Activated Akt phosphorylates human eNOS at serine 1177 and subsequently increases NOS activity. Because Akt and eNOS are both localized in the bladder urothelium, phosphorylation of eNOS by Akt provides an attractive mechanism for rapid increases in urinary NO production. Female mice were intraperitoneally injected with LPS (25 mg/kg) or pyrogen-free water (control). Four hours before LPS injection, some mice were injected with wortmannin, which inhibits Akt phosphorylation. Levels of urinary cyclic GMP, a downstream product of NO, increase 75% within 1 h after intraperitoneal injection of LPS, and this increase is blocked by wortmannin. Bladder eNOS and phosphorylated eNOS protein increase 94 and 151%, respectively, 1 h after LPS treatment, whereas iNOS was not detected. Wortmannin decreases eNOS phosphorylation by 60%. Furthermore, bladder 
Ca^{2+}-dependent NOS activity (eNOS, neuronal NOS) is increased 79 ± 20% 1 h after LPS treatment, whereas there is no increase in 
Ca^{2+}-independent (iNOS) activity (n = 4). Increases in urinary cyclic GMP, NOS activity, and eNOS protein and phosphorylation 1 h after induction of inflammation with LPS, indicate that eNOS plays a role in the early response to bladder inflammation.

The number of uncomplicated Escherichia coli-induced urinary tract infections (UTIs) per year is 6 to 8 million in the United States and 130 to 175 million globally. Antimicrobial resistance has increasingly developed among E. coli strains causing UTIs (Russo and Johnson, 2003), making understanding of host defense during UTIs of primary importance. In rodent models of UTIs in which E. coli lipopolysaccharide (LPS) is intravesically instilled or intraperitoneally injected (Olsson et al., 1998; Saban et al., 2001 Wheeler et al., 2001), induction of cyclooxygenase-2, cytokines, and inducible nitric-oxide synthase (iNOS) along with urothelial exfoliation occur within 4 to 24 h. Induction of inflammation by LPS or E. coli in bladder uroepithelial cells involves the Toll-like receptors and CD14 (Schilling et al., 2003). These activate signaling pathways, including nuclear factor-κB and p38 mitogen-activated protein kinase, with subsequent production of interleukin-6. We have identified another signaling pathway in mouse bladder, the phosphorylation of Akt/protein kinase B (Akt) by phosphatidylinositol 3-kinase (PI-3-kinase), which is up-regulated within 30 min of LPS treatment (Tamarkin et al., 2003). In addition to its role in regulating cell survival and growth signals, phosphorylation of Akt, a serine/threonine kinase, can directly phosphorylate bovine endothelial NOS (eNOS) at serine 1179 and increase NOS activity severalfold in endothelial cells (Fulton et al., 1999; Cirino et al., 2003). Chronic overexpression of eNOS reduces LPS-induced hypotension, lung injury, and death, indicating a protective role for eNOS in septic shock. The colocalization of Akt and eNOS in bladder urothelium, and the rapid phosphorylation of Akt in the bladder after LPS treatment (Tamarkin et al., 2003) may indicate a role for eNOS phosphorylation in the early inflammatory response to LPS.

Three isoforms of NOS have been described in the urinary tract: neuronal NOS, iNOS, and eNOS. NO production by the...
constitutive, Ca\(^{2+}\)-dependent nNOS causes nonadrenergic, noncholinergic relaxation in urethral smooth muscle (Dokita et al., 1991). NO production is dramatically increased by iNOS in bladder inflammation and infection (Wheeler et al., 1997; Olsson et al., 1998; Poljakovic et al., 2001). eNOS is the particulate, Ca\(^{2+}\)-dependent isofrom of NOS, which traditionally is considered a constitutive enzyme. There is a high degree of inter- and intracellular specificity of eNOS expression. eNOS is expressed primarily in the caveolae of epithelial and endothelial cells (Shaul, 1997), in mucosal umbrella cells, and in submucosal capillaries of normal adult human urothelium (Shochina et al., 2001); however, its role in urinary tract function is not well described. Recently, an appreciation has grown for the ability of post-translational modifications to regulate eNOS activity. Factors including shear stress, autocorticoids, or growth factors have been hypothesized to induce eNOS or to shift it to a more active state. Dynamic subcellular targeting, protein-protein interactions, and phosphorylation of eNOS through the PI-3-kinase-Akt pathway modulate NO production by eNOS (Cirino et al., 2003).

Because up-regulation of iNOS requires transcription and translation, phosphorylation of eNOS by Akt provides an attractive mechanism for rapid increases in NO production in LPS-treated mice. Mice were treated with an intraperitoneal (i.p.) injection of LPS. LPS increased levels of urinary cyclic GMP, the downstream product of NO (Olsson et al., 1998), within 1 h of LPS injection. We then localized both Akt and eNOS in bladder urothelium. eNOS and phospho-eNOS but not iNOS proteins in the bladder were up-regulated within 1 h after LPS injection. Because iNOS is the Ca\(^{2+}\)-calmodulin-independent isofrom of NOS, whereas the constitutive isoforms nNOS and eNOS are Ca\(^{2+}\)-calmodulin-dependent, NO activity was monitored in the presence and absence of EGTA. Only constitutive NOS activity was elevated 1 h after LPS treatment. Wortmannin, a PI-3-kinase inhibitor, reduces Akt phosphorylation in the bladder (Tamarkin et al., 2003). Wortmannin treatment of LPS-treated mice reduced eNOS phosphorylation. Thus, we determined that eNOS is responsible for early increases in NO production in bladder inflammation.

Materials and Methods

Induction of Inflammation. Female C57BL/6 mice (8–12 weeks old) were injected with i.p. LPS (25 mg/kg) or with i.p. pyrogen-free water (controls) as per our institutionally approved animal use protocol in accordance with the Guide for the Care and Use of Laboratory Animals. For a single determination (n), two to three mouse bladders were pooled. Each experiment was replicated at least four times, for a total of at least eight bladders per experimental condition. LPS was prepared by trichloroacetic acid extraction from E. coli, serotype 0127:B8 (Sigma-Aldrich, St. Louis, MO). Thirty minutes to 2 h after injection the animals were sacrificed, and their bladders and hearts were removed and placed in ice-cold PBS containing phosophatase inhibitors (5 mM sodium fluoride, 1 mM sodium pyrophosphate, and 1 mM sodium vanadate). In a second set of experiments, mice were intraperitoneally injected with wortmannin (1.4 mg/kg) in pyrogen-free water containing 2% methanol (vehicle) or with vehicle alone (4–6 mice/group). Four hours after the initial treatment, all mice were injected with i.p. LPS (2–3 mice/group). After 1 h, the mice were sacrificed and their bladders and hearts were processed as described above. Before any injection and before sacrifice, urine was collected from mice by gentle manipulation of the abdomen.

Immunohistochemistry of Bladders. After deparaffinization and rehydration of paraformaldehyde-fixed, slide-mounted bladder tissue sections, antigens were unmasked by heating in 10 mM sodium citrate, pH 6.0 (10 min, 95°C). Endogenous peroxide was quenched using 1% hydrogen peroxide, and tissues were blocked with 5% goat serum. Tissues were then incubated with Akt, phospho-Akt (serine)-specific polyclonal antiserum (Cell Signaling Technology Inc., Beverly, MA) and eNOS-specific monoclonal antiserum (BD Biosciences Transduction Laboratories, Lexington, KY) overnight. After washing with PBS, samples were incubated with the appropriate biotinylated goat anti-rabbit or goat anti-mouse IgG conjugated (Jackson ImmunoResearch Laboratories Inc., West Grove, PA). Staining was performed using the avidin, biotinylated horseradish peroxidase complex (Vector Laboratories, Burlingame, CA). The slides were then washed, dehydrated, and hematoxylin counterstained. For negative controls, the primary antibody was omitted from the incubating sera.

Measurement of Urinary Cyclic GMP Levels. Control urine was collected from mice before any treatment or after treatment with pyrogen-free water. Other urine was collected after completion of treatment. Cyclic GMP levels were determined in 1:50 dilutions of urine supernatants using an INOS-specific radiolmmunoassay (Biomedical Technologies, Stoughton, MA) (Smith et al., 1996; Olsson et al., 1998).

Myeloperoxidase activity was measured in urine or in myeloperoxidase standards (2.5–12.5 mU/ml) with freshly prepared 1 mM O-dianisidine HCl in 100 mM KH\(_2\)PO\(_4\) (pH 6) plus 0.05% H\(_2\)O\(_2\). After 10 min, absorbance was read at 450 nm using a microplate reader (DYNATECH Labs, Chantilly, VA).

Immunoblot Analysis. For partial purification of NOS before immunoblot analysis, we used the method of Liu and Sessa (1994). Mouse bladders and hearts were homogenized in lysis buffer (20 mM HEPES, 315 mM sucrose, 1% glycerol, 1% Nonidet P-40 detergent, 0.05 mM phenylmethylsulfonyl fluoride, protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO), and phosphatase inhibitors (pH 7.45)). Homogenates were incubated with gentle rocking (2 h, 4°C), centrifuged (20,000 g, 20 min, 4°C), and then an aliquot was removed (total cell lysate). The supernatants were incubated (2 h, 4°C) with 2′,5′ ADP-Sepharose beads (Pharmacia AB, Uppsala, Sweden), centrifuged, and the resultant supernatant was removed. Laemmli sample buffer was added to the total cell lysate and to 2′,5′ ADP-Sepharose beads containing NOS proteins. All samples were then heated (95°C, 5 min). To normalize the amount of protein loaded, Western blotting was performed with actin antiserum (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Blots were scanned to determine the relative intensity of the actin bands, and amounts loaded in subsequent gels were normalized to the amount of actin in each sample. For NOS proteins, equivalent amounts of protein were separated by electrophoresis using SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and blotted with iNOS-specific polyclonal antiserum (Affinity Bioreagents, Golden, CO), eNOS-specific monoclonal antiserum (BD Biosciences Transduction Laboratories), and phosphorylated eNOS (Ser1177, human)-specific antiserum (Cell Signaling Technology Inc.). Bovine aortic endothelial cells treated with vascular endothelial growth factor were used as a positive control for phosphorylated eNOS. Akt and phosphorylated Akt (Ser473) were measured in total cell lysates using appropriate antibodies (Cell Signaling Technology Inc.). Appropriate horseradish peroxidase conjugated secondary antibodies (Jackson ImmunoResearch Laboratories Inc.) were used followed by detection of immunoreactive proteins with the enhanced chemiluminescence method (Amersham Biosciences UK, Ltd., Buckinghamshire, Little Chalfont, UK). All bands were quantified using EDAS software (Eastman Kodak, Rochester NY). Phosphorylated eNOS and Akt were normalized to total eNOS and Akt, respectively.
**Assay of NOS Activity.** Mouse bladders were minced and suspended in 500 μl of ice-cold homogenization buffer [50 mM Tris-HCl, 0.1 mM EDTA, 1.0 mM dithiothreitol, 1% (v/v) Nonidet P-40, 0.1% SDS, and 0.1% deoxycholate, 0.05 mM phenylmethylsulfonyl fluoride, protease inhibitor cocktail, and phosphatase inhibitors, pH 7.5]. Bladders were homogenized (10 s, at 70% power, three times, and 20 s at 50% power) using a Polytron (Brinkmann Instruments, Westbury, NY). Tissue homogenates were incubated on ice (10 min) and centrifuged (10,000 g, 10 min, 4°C). The particulate fraction was rehomogenized (0.5 ml of homogenization buffer), and both supernatant and rehomogenized particulate fractions were incubated on ice (10 min) followed by centrifugation (50,000 g, 20 min). Supernatants were combined and applied to a 0.5-ml column of AG 50W-X8, Na+ form (Bio-Rad, Hercules, CA) to remove endogenous L-arginine.

NOS activity was then measured in these solubilized preparations as the formation of [14C]L-citrulline from [14C]L-arginine (DuPont, Wilmington, DE). The bladder enzymes were incubated (37°C, 45 min) with HEPES buffer containing [14C]L-arginine (0.2 μCi, 3 μM), 10 mM valine, 2 mM NADPH, 10 μM tetrahydrobiopterin, 10 μM FAD, and protease inhibitor cocktail (Olsson et al., 1998). Activity was measured in the presence of 0.2 U of calmodulin-1 mM CaCl2. Activity was inhibited with either 2 mM Nω-nitro-l-arginine methyl ester to measure total NOS activity, or with 5 mM EGTA to measure constitutive NOS activity. The assay was terminated by the addition of 1.0 ml of 20 mM HEPES (pH 5.5) containing 2 mM EDTA and 20 μM l-arginine. The sample was then applied to a 1.0-ml column of AG 50W-X8, Na+ form and eluted with 1.0 ml of 20 mM HEPES (pH 5.5). The radioactivity of the column effluent was measured in a liquid scintillation counter, after addition of 15 ml of Optifluor (PerkinElmer Life and Analytical Sciences, Boston, MA). Before measurement of protein levels, particulate samples were hydrolyzed with 1 N NaOH. Protein concentrations were measured (DC assay; Bio-Rad) with bovine γ-globulin as a standard.

**Statistics.** Data are presented as mean ± S.E.M. Differences between urine treatment groups are determined by analysis of variance followed by Fisher’s protected least significant difference. Differences between protein samples are determined by the paired t test (Statview; SAS Institute, Cary, NC).

**Results**

**Hematoxylin Staining and Immunohistochemical Localization of Akt and eNOS.** One-hour LPS treatment did not increase inflammatory cell numbers compared with control bladders. Akt (data not shown), phosphorylated-Akt, and eNOS were colocalized in bladder urothelium by immunohistochemistry (Fig. 1).

**Effect of LPS Treatment on Urinary Cyclic GMP and Myeloperoxidase Levels.** Urinary cyclic GMP levels are increased 75% in mice treated with LPS for 1 h compared with control mice. Wortmannin decreased urinary cyclic

![Fig. 1. Immunohistochemical localization of phospho-Akt and eNOS in mouse bladder (400×). A, PBS control for phospho-Akt (polyclonal goat anti-rabbit biotinylated conjugate only). B, PBS control for eNOS (monoclonal goat anti-mouse biotinylated conjugate only). C, bladder stained with polyclonal anti-phospho-Akt. D, bladder stained with monoclonal anti-eNOS. u, urothelium.](http://jpet.aspetjournals.org/doi/10.1124/jpet.198.7.454)

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![Fig. 2. Effect of LPS and wortmannin on urinary cyclic GMP levels. Cyclic GMP is measured in the urine of control mice, mice treated with LPS for 1 h, mice treated with wortmannin for 4 h followed by pyrogen-free water, and mice treated with wortmannin (4 h) followed by LPS (1 h). n, number of mice. * p < 0.05 compared with control value.](http://jpet.aspetjournals.org/doi/10.1124/jpet.198.7.454)
GMP levels in control and LPS treated mice (60 min) (Fig. 2). Urinary myeloperoxidase levels did not change in these groups of LPS or wortmannin-treated mice compared with untreated mice. Urinary levels of cyclic GMP remained elevated in mice treated for 4 to 18 h with LPS.

**Western Blot Analysis of eNOS, Phosphorylated-eNOS, and iNOS.** One h after LPS treatment, eNOS and phosphorylated eNOS protein were increased 94 and 151% \((n = 4)\), respectively, over control bladder values (Figs. 3 and 4). The phosphorylation of eNOS in the heart after 1-h treatment with LPS was increased by 81 ± 45% compared with controls (Fig. 4). iNOS was not detected (data not shown) in the 1-h LPS-treated mice.

Treatment with wortmannin 4 h before a 1-h LPS treatment reduced Akt phosphorylation by 63%. Relative phosphorylation of eNOS in mouse bladders treated with wortmannin before i.p. LPS was reduced 44% compared with mouse bladders treated with vehicle before i.p. LPS (Fig. 5). The reduction of eNOS phosphorylation by wortmannin in control bladders was not significant \((n = 4)\). eNOS protein levels in mouse bladders first treated with vehicle before i.p. LPS were not different from mouse bladders treated with wortmannin and then i.p. LPS (Fig. 5).

**Increases in NOS Activity after LPS Treatment.** LPS treatment of mouse bladders increased \(\text{Ca}^{2+}\)-dependent NOS activity (a measure of combined eNOS and nNOS activity) from 9.8 ± 1.9 to 18.6 ± 2.3 pmol citrulline/min/mg protein \((n = 5\) experiments) within 1 h. Thus, LPS induced a 90% increase in constitutive, \(\text{Ca}^{2+}\)-dependent activity 1 h after LPS treatment. \(\text{Ca}^{2+}\)-dependent NOS activity, in either control or LPS-treated mouse bladders, was not significantly different from total NOS activity (Fig. 6). LPS did not increase \(\text{Ca}^{2+}\)-independent activity (a measure of iNOS), and this activity represented only 5.6% of total NOS activity 1 h after LPS treatment \((n = 5\) experiments).

**Discussion**

The goal of this study is to show that phosphorylation of eNOS, a post-translational modification, can rapidly increase NO production in the bladder. One hour after treatment with i.p. LPS, urinary cyclic GMP, eNOS protein and phosphorylation, and constitutive bladder NOS activity are all increased. This indicates that eNOS phosphorylation along with increases in eNOS protein may be involved in the early response to bladder inflammation. Increases in NO production during urinary tract infections (Wheeler et al., 1997), Bacille Calmette-Guerin treatment of bladder cancer (Jansson et al., 1998), and transplant rejection (Smith et al., 2000) previously have been linked to iNOS activation. In mouse and rat models where inflammation was induced with LPS, up-regulation of iNOS in the bladder occurs after 4 h (Olsson et al., 1998; Saban et al., 2001; Poljakovic et al., 2001). After 1 h of LPS treatment, there is no increase in the number of inflammatory cells stained with hematoxylin or in urinary myeloperoxidase, a specific marker for neutrophils, com-

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**Fig. 3.** Western blot analysis of eNOS after LPS treatment of bladder and heart. A, representative eNOS Western blots of bladder and heart after treatment with pyrogen-free H\(_2\)O (control) or LPS for 1 h. Duplicate samples are shown for H\(_2\)O- and LPS-treated bladder. B, analysis of relative amounts of eNOS protein in bladders at 1 h \((n = 10)\) and 2 h \((n = 4)\), relative to control (pyrogen free H\(_2\)O, \(n = 14\)). \(*p < 0.05\) compared with control value.
pared with controls. The static number of inflammatory cells after 1 h of LPS treatment indicates that changes in eNOS occur in urothelial cells and are not due to an influx of inflammatory cells that occurs 4 h after LPS treatment in a rat UTI model (Olsson et al., 1998).

Increases in urinary cyclic GMP reflect an increase in NOS, which may occur in the bladder as well as in other tissues. In the rat model of bladder inflammation, intravesical instillation of LPS increases urinary nitrate/nitrite levels to the same level as intraperitoneal injection of LPS (Wheeler et al., 2001). Intravesical instillation of LPS does not increase NOS activity or iNOS mRNA in either the heart or the kidney (Olsson et al., 1998). Therefore, increases in NOS activity may be due in a large part to increases of NOS in the bladder. Furthermore, increases in constitutive bladder NOS activity in the mouse model of inflammation reflect up-regulation of eNOS phosphorylation and protein, which precedes induction of iNOS.

In humans (Shochina et al., 2001) and in mice (Burnett et al., 1997), eNOS is localized in bladder urothelium. We have recently shown that Akt, a signaling protein activated by PI-3-kinase, can directly phosphorylate eNOS and is also localized in mouse urothelium. Akt phosphorylation in mouse bladder is evident within 30 min of treatment with LPS and increases for 2 h. Wortmannin, a specific inhibitor of PI-3-kinase, blocks LPS-induced Akt phosphorylation in mouse bladder (Tamarkin et al., 2003) and also blocks LPS-induced eNOS phosphorylation. The reduction in eNOS phosphorylation in the bladder and urinary cyclic GMP after wortmannin treatment indicates that the PI-3-kinase/Akt pathway is involved in the early response to LPS-induced inflammation.

Increases in NO in response to shear stress, estradiol, insulin-like growth factor, and vascular endothelial growth factor have been linked to eNOS phosphorylation by Akt in endothelial cells (Shaul, 2002). In a whole animal model of portal hypertension, eNOS phosphorylation and NO production are up-regulated in the superior mesenteric artery (Iwakiri et al., 2002). Both electrical stimulation of the cavernous nerve and direct intracavernosal injection of papaverine, a vasorelaxant drug, cause rapid (after 15 s) increases in the phosphorylation of Akt and eNOS. This phosphorylation is reduced by wortmannin and by LY294002, another inhibitor of PI-3-kinase. Both wortmannin and LY294002 reduce erections. Although NO production is rapidly increased by stimulation of nNOS, eNOS may be responsible for sustained NO production and maximal erection (Hurt et al., 2002).

In addition to LPS-induced increases in eNOS phosphorylation, we also noted increases in eNOS protein in bladder 1 h after i.p. injection of LPS. This increase in eNOS protein was not changed by treatment with wortmannin. Several lines of evidence, in addition to data presented in this manuscript, point to the up-regulation of eNOS as an early event in inflammatory processes of the bladder urothelium as well as of the gastric mucosa. In rats, eNOS and iNOS mRNA and protein along with eNOS immunoreactivity in the urothelium are increased 2 h after BCG transurethral injection (Oh et al., 2001). In urinary bladder tumor biopsy specimens, Ca²⁺-dependent NOS activity was several-fold higher in BCG-treated bladder, compared with normal bladder wall and bladder tumor. eNOS immunoreactivity is detected in urothelial cells and in BCG-treated bladder mucosa (Jansson et al., 1998). In rat gastric mucosa, increases in both eNOS
mRNA and iNOS mRNA are noted 1 h after i.p. injection of LPS. At 3 and 5 h after i.p. LPS, iNOS but not eNOS remains elevated. Evaluation of gastric luminal nitrate and nitrite levels, another measure of NO, is also increased after 1 h of LPS treatment. Unlike the bladder, however, up-regulation of NO may be due to increases in iNOS because Ca\(^{2+}\)-independent, but not Ca\(^{2+}\)-dependent NOS activity, is elevated in gastric mucosa after 1 h of LPS treatment (Helmer et al., 2002). In the mouse bladder, after 1 h of treatment with LPS, we found an increase in Ca\(^{2+}\)-dependent but not Ca\(^{2+}\)-independent NOS activity. The mechanism for up-regulation of eNOS protein in urothelium has not been elucidated. In endothelial cells, increases in eNOS protein by a variety of stimuli are associated with increases in protein synthesis, mRNA levels (Zembowicz et al., 1995) or mRNA half-life (Laufs and Liao, 1998). In endothelial cells, however, the up-regulation of eNOS occurs after 3 h or more.

Although eNOS has been localized to the urothelium, its function in the bladder has not been delineated. The role of NO produced by eNOS in septic shock is seen as protective compared with the deleterious effect of NO produced by iNOS. LPS injection in mice overexpressing eNOS causes significantly less mortality than LPS injection in mice that do not overexpress eNOS. When treated with LPS, mice that chronically overexpress eNOS in their endothelium were resistant to LPS-induced hypotension, lung injury, and death (Yamashita et al., 2000). In the urinary tract, induction of eNOS protein and phosphorylation may play a role in the pathophysiology of inflammatory processes in the bladder. PI-3-kinase is involved not only in the bladder response to LPS but also in bacterial adherence and invasion induced by type 1 pilus adhesin (FimH) encoded by uropathogenic E. coli (Martinez and Hultgren 2002). Because PI-3-kinase-Akt activation induces phosphorylation of eNOS, bacterial adherence and invasion may also involve eNOS. Both eNOS and iNOS mRNA are detected in the urine from subjects with UTIs (Wheeler et al., 1997). Increases in NO production during BCG treatment of subjects with bladder cancer may be linked to eNOS as well as iNOS (Jansson et al., 1998). Urinary NOS activity and cyclic GMP levels are decreased in subjects with interstitial cystitis compared with control subjects (Smith et al., 1996; Erickson et al., 2002), perhaps indicating that eNOS is down-regulated in this patient population. Because the NO produced by eNOS is believed to be beneficial, the role of phosphorylation of eNOS by PI-3-kinase in up-regulating NO production during UTI and during BCG treatment of subjects with bladder cancer, and its role in the down-regulation of NOS in interstitial cystitis, should be subjected to further investigation.

References


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