A review of the L-arginine – nitric oxide – guanylate cyclase pathway as a mediator of lower urinary tract physiology and symptoms

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Lower urinary tract symptoms (LUTS) are common and costly conditions that affect millions of men and women worldwide. A focal area of research into the cause and potential treatment of LUTS is the nitric oxide pathway, which is involved in nerve-induced relaxation in the lower urinary tract. Isoforms of NOS, including nNOS, eNOS, and iNOS, have been identified in the lower urinary tract of both animals and humans. Nerves that are immunoreactive to nitric oxide synthase (NOS) mainly serve the bladder outlet region, but some serve the detrusor. Pathology of the l-arginine-nitric oxide-cGMP pathway involving nNOS and eNOS may lead to impaired relaxation of the urethral outlet, increased bladder afferent activity, and detrusor smooth muscle overactivity. Such pathology has been implicated in the conditions of detrusor instability, urinary incontinence and outlet obstruction. iNOS may play an important role in inflammatory and infectious conditions of the bladder. Strategic manipulation of nitric oxide (NO), or interventions that address its mechanisms of action, possibly by pharmacological means or with gene therapy, may restore function or produce desired functional effects in the lower urinary tract.

Key Words: nitric oxide, bladder, urethra, urinary frequency, urinary incontinence, urinary obstruction

Introduction

The nitric oxide (NO) pathway is an area of interest in urology because of its role in penile erection and the drug therapies that successfully treat erectile dysfunction by targeting the pathway. These drugs evolved from experimental and clinical work, which demonstrated that NO released from nerve ends relax the vascular and corporal smooth muscle cells of the penile arteries and trabeculae respectively to induce penile erection.

Recently there has been increased interest in how NO may be involved in the physiologic mechanisms of the lower urinary tract. This article reviews the current knowledge of the NO biochemical pathway, the distribution and potential target tissues in the lower urinary tract, and existing and potential therapeutic strategies for the treatment of lower urinary tract symptoms (LUTS) resulting from abnormalities in the NO pathway.

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conducted for the period 1966 to 2002 using key words nitric oxide, urinary tract, incontinence, estrogen, bladder, urethra, infection and obstruction. The first relevant references appeared in 1992.[1-3]

The L-arginine-nitric oxide-cGMP pathway

Physiologically, nitric oxide is produced by the enzyme nitric oxide synthase (NOS). Three forms of NOS have been identified: nNOS, eNOS, and iNOS, which are produced by the human genes NOS1 (for nNOS), NOS2 (for iNOS), and NOS3 (for eNOS). The nomenclature for NOS subtypes reflects the source of the original isolates: neuronal tissues for nNOS; immunoactivated macrophage cell lines for iNOS, and vascular endothelium for eNOS. The role of all forms of NOS is to produce NO, but the triggers and regulators of the process vary widely and thus NOS plays many roles in human biology, ranging from homeostasis to regulation of the immune system.[4] Importantly, the subtypes are now known not to be limited to the tissue sites from which they were first isolated and the same NOS subtype may play different biological roles in different tissues.[4,5]

nNOS and eNOS have important general biochemical features in common[4] and are referred to as "constitutive forms". They are both calcium-dependent, require calmodulin and reduced nicotinamide adenine dinucleotide phosphate (NADPH) for catalytic activity, and are competitively inhibited by arginine derivatives. The nNOS and eNOS subtypes act in the biochemical cascade that targets the cGMP pathway. As indicated by their names, nNOS and eNOS are involved in the regulation of neurotransmission and blood flow respectively.

Unlike nNOS and eNOS, iNOS is calcium-independent and is termed "inducible". iNOS is induced by the inflammatory reaction and is important because it produces nitrogenous amines as end products. This subtype is of particular interest in the urinary tract because it is known to be associated with the carcinogenic process involving transitional epithelium.[6,7] iNOS is primarily expressed in inflammatory macrophages and appears to be involved in the cytotoxicity of macrophages.[8] It produces NO along with its oxidative byproducts, nitrate and nitrite.

Regardless of the specific isoform, NOS produces NO by oxidation of L-arginine. L-arginine is one of the basic amino acids. It circulates in the blood at a concentration of about 100 micromoles/L.[9] It is also found inside cells, either synthesized from the urea cycle or obtained from oral ingestion.[9] The concentration of L-arginine inside the cells far exceeds that of the plasma, and thus is generally available in abundance in the cell. L-arginine has complex actions in the immune system and is involved in wound healing.[10] Inside the cell, NOS catalyzes the oxidation of L-arginine to NO and L-citrulline. This reaction requires molecular oxygen and NADPH as co-substrates, along with many other molecules as cofactors. There are endogenous blockers of the cascade at this level that have been identified in humans. For example, N^G-N^G-dimethylarginine (ADMA) is an endogenous blocker of NOS that is elevated in patients with renal failure.[11]

Once produced, the gaseous NO acts as a neurotransmitter or paracrine messenger. It has a very short biologic half-life: only 5 seconds.[12] NO may act within the cell or diffuse and interact with nearby target cells. The specific type of cell targeted differs depending on where the NO is produced. NO activates soluble guanylate cyclase, leading to the formation of cyclic GMP from guanicine 5′ triphosphate,[13,14] increasing levels of cGMP. cGMP then acts through a variety of enzymatic reactions that may involve protein kinases, phosphodiesterases or modulation of ion channels.

In the lower urinary tract, NO has various target cells, including detrusor smooth muscle cells, striated muscle involved in the urinary sphincter mechanism, interstitial cells and uroepithelial cells in the bladder, and vascular smooth muscle cells in the urethra Figure 1.

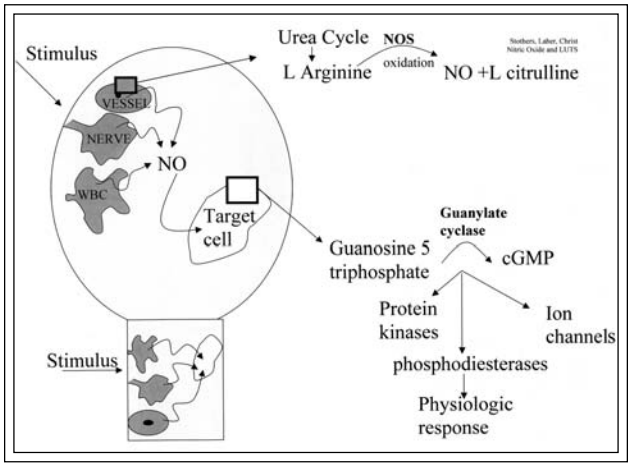

Figure 1. The NOS cascade in the lower urinary tract. The stimulus is likely different for each type of NOS-containing cells .

Distribution and function of NOS in the lower urinary tract

Mapping techniques

In the lower urinary tract, the location of nNOS has been the focus of numerous reports. Mapping of nNOS in the peripheral nerves has been done primarily in animal models, with only recent work on human tissue. Two methods have been used to localize NOS in tissues: indirectly by measuring the levels of products of NOS activity, such as the L-citrulline;[15] and directly by immunocytochemistry. Immunocytochemistry, which uses vital stains, is able not only to determine the location of NOS within the cells, but also to determine the quantity and differentiate the specific isoforms of NOS present.[16]

Animal models

The density of NOS-containing nerves in the lower urinary tract differs between locations. Most NOS-location investigations have been carried out on male animals, including pigs,[17] rabbits[18] and guinea pigs.[19,20] The highest density of NOS-containing nerves has been localized at the level of the bladder neck and urethra, with lower density found throughout the bladder body.[17,19,20] It has become clear that the distribution of nNOS nerves is species-specific[21] and may also be gender dependent.[22] In addition to species and gender differences in NOS, cGMP levels may be age dependent.[23,24] Thus, there is a need to map nNOS density in both male and female human subjects across a wide range of ages.

Animal studies have shown that NO is involved in a concentration-dependent fashion in urethral relaxation and contraction,[25-28] and in control of urethral outlet activity.[29,30] NO is also involved in the micturition reflex[30,31] and appears to influence both sphincter and detrusor activity.[32]

Human data

To date, there have been six reports in the literature on the distribution of NOS in the human lower urinary tract,[33-38] with the greatest emphasis on nNOS. It has been determined that NOS detection methods are reliable in cadaveric specimens up to 48 hours following death.[39] Since living human urethral tissue is difficult to obtain, a variety of human tissue sources have been used for substrate, including: bladder biopsy specimens; gross specimens of bladder and urethra at the time of radical cystectomy or radical prostatectomy; fresh cadaveric specimens; tissue retrieved at the time of organ retrieval for transplantation; and fresh surgical specimens of males undergoing trans-sexual reassignment.

Male human subjects

In a 1994 study that used indirect measures of NOS activity,[35] nNOS activity was found to be greatest in the prostatic urethra and the bladder neck, with lesser activity in the detrusor. Guanylate cyclase levels had a similar distribution. Another study used antibody staining of cold cup biopsy specimens, surgical specimens and fresh cadaveric tissue[34] to identify the primary target cells of NO in the lower urinary tract. There were regional differences in the distribution of cGMP in the bladder, bladder neck and urethra: cGMP concentration was high in the interstitial cells in the detrusor muscle, uroepithelial cells, and vascular smooth muscle cells. It was concluded that these cells are the targets of NO in the lower urinary tract. It was also suggested that the target in the urethra is the smooth muscle cell, and the target in the bladder is the interstitial cell. nNOS has also been localized to, and implicated in the control of, striated muscle in the male human membranous urethra.

Female human subjects

Few studies have been done on female subjects. In Smet's study,[34] only one of 14 subjects was female. Only two studies describe the distribution of NOS at the level of the bladder outlet in females.[33,38] In 1999, Ho et al[33] described the gender differences in concentration of nNOS and heme-oxygenase-2 (HO-2) in urethral smooth muscle. While nNOS and HO-2 were found in the urethra of both genders, there was a difference in the distribution between males and females. In the females, HO-2 was confined to the proximal and middle third of the urethra.

Recently, NOS isoforms have been described in the human bladder from 18 patients of unspecified gender with superficial bladder cancer.[40] Benign biopsy material was obtained at the time of transurethral resection of the tumors. Detrusor smooth muscle and urothelium were found to contain eNOS, although the density in the urothelium was much lower. nNOS was found in nitrergic fibers located submucosally and between muscle cells. Detrusor, vascular smooth muscle, interstitial cells, nerve fibers and transitional epithelium contained soluble guanylate cyclase, and were therefore identified as potential target cells.

Based on the distribution of NOS in the urinary tract, eNOS and nNOS are likely indirectly involved in the regulation of the bladder smooth muscle contraction/relaxation cycle, neurogenic responses and blood flow. There is some evidence that iNOS is involved in the production of bladder symptoms associated with inflammatory and infectious conditions of the bladder.[41]

Role of the L-arginine-NO-cGMP pathway in LUTS

It is clear that the lower urinary tract has a rich supply of nitrergic nerves and that NO is involved in the nonadrenergic, noncholinergic (NANC)-mediated cascade of events that control lower urinary tract storage and emptying. Experimental work has shown that NO at the level of the bladder is involved in bladder relaxation and that decreased release of NO results in increased bladder activity.[42] Thus, in humans, pathology in the NO pathway may lead to clinical symptoms of urinary frequency and decreased functional volumes.

There are several points in the NO pathway, or NOS cascade, at which pharmaceutical or naturopathic intervention may have therapeutic effect in the urogenital system. The most obvious recent example of how alterations in the NOS cascade may be clinically useful is the success of sildenafil citrate for the treatment of erectile dysfunction. Sildenafil is a selective phosphodiesterase type 5 (PDE5) inhibitor. In the penis, PDE5 is the predominant enzyme responsible for cGMP hydrolysis. In LUTS, it may also be desirable to increase NO in certain conditions, whereas a reduction of NO may be beneficial in other situations.

For example, increased activity of NOS (release of NO) at the level of the urethra is associated with increased urethral relaxation. Therefore, selectively increasing urethral NOS may be beneficial in management of obstruction of the lower urinary tract in which increased urethral relaxation and decreased urethral pressure are desired. Increased NOS activity may act as a bladder relaxant and could therefore be beneficial in management of overactive bladder.

Conversely, lower levels of urethral NOS may be beneficial in increasing urethral pressure and could theoretically be beneficial in treatment of stress urinary incontinence. Down-regulation of NOS in the bladder could be linked to conditions like detrusor instability but may also be beneficial for disorders characterized by detrusor hyporeflexia.

Potential ways of altering NO levels include:

- direct administration of NO as a gas
- administration of NO donors
- administration of NO agonists
- preservation of cGMP
- lowering of endogenous NOS inhibitors
- administration of exogenous NOS activators
- increasing substrate for NO synthesis

Direct administration of NO as a gas

While NO is present in the exhaled air of animals and humans, and it can be used safely inhaled (as in the treatment of ARDS), no specific work has been done on the effects of inhaled NO on the urinary tract. This may be due to the fact that when inhaled systemically as a gas, NO is inactivated in the blood by reaction with oxyhemoglobin.

Administration of NO donors

A number of compounds serve as NO donors, including organic nitrates (e.g. glyceryl trinitrate (GTN)), nitrites (e.g. amyl nitrite), inorganic nitroso compounds (e.g. sodium nitroprusside (SNP)), sydnonimines (e.g. molsidomine, SIN-1) and S nitrosothiols. All NO donors exert their action after their metabolism into NO (hence the nomenclature NO donor). NO donors are traditionally used for cardiovascular conditions to exploit their dilator action on arterial and venous smooth muscle and their ability to increase coronary flow. GTN, given to women in preterm labor, causes a reduction in uterine contractility and prolongs pregnancy.[43] SNP releases NO spontaneously whereas sydnonimines generate NO after reacting with molecular oxygen.[44] Potential drawbacks include tolerance to NO at the level of guanylate cyclase and inhibition of endogenous synthesis of NO with chronic administration of NO donors.[44]

Administration of NO agonists

Increasing levels of NO agonists may augment the generation of endogenous NO. Agonists that cause long term activation of the L-arginine NO pathway already exist. For example, angiotensin-converting enzyme (ACE) enhances the production of NO in endothelial cells.[45]

Preservation of cGMP

There are a number of phosphodiesterase isoenzymes, some of which only hydrolyze cGMP (type 5). Type 5 PDEs are currently in clinical trials for male and female sexual dysfunction.[46-48] Their utility for treatment of bladder dysfunction is undocumented.

Lowering of endogenous NOS inhibitors

Analogs of L-arginine exist that are competitive and sometimes irreversible inhibitors of NOS. Endogenous compounds of asymmetric dimethylarginine (ADMA) and N^G monomethyl L-arginine (LNMMA) are present in human plasma and urine and accumulate in the plasma of patients with chronic renal failure.[49] They have been suggested as contributing to the hypertension and white blood cell dysfunction of renal failure.[11]

Administration of exogenous NOS inhibitors

Methylene blue is a guanylate cyclase inhibitor.[50] Another inhibitor used in basic science work is 1H-[1,2,4]oxadiazolo [4,3-a]quinoxaline-1-one (ODQ).[31]

Increasing substrate for NO synthesis

While intracellular concentrations of L-arginine are even greater than plasma concentrations, and thus it is assumed that there is an abundance of the substrate, researchers have suggested that oral supplementation of L-arginine may increase levels of NO. Investigators have tried oral supplementation in various disorders with variable results. For example, Chen et al[51] administered oral L-arginine to 50 men with organic erectile dysfunction and found significant subjective improvement in symptoms. Naturopathic supplements of arginine are readily available. Reported side effects of arginine supplementation include nausea, diarrhea, headache, flushing, numbness and hypotension.[52,53] Delayed menses and night sweats observed as side effects of L-arginine in one study[54] may have been related to arginine-stimulation of prolactin from the pituitary gland. There has been one report of a death following accidental overdose of l-arginine in a 21-month old girl.[55]

Another possible substrate is NO hydroxy-L-arginine.[56]

The Role of NO in bladder outlet obstruction

Changes in the function of the bladder secondary to anatomic obstruction include instability of the bladder, increased bladder pressure with voiding, and thickening of the detrusor muscle. These changes manifest in slowing of the urinary stream, frequent urination and increased residual urine. Proposed pathophysiology related to these symptoms includes: denervation changes in the detrusor wall; changes in peptide content in the peripheral bladder nerves and changes in the response of the detrusor smooth muscle to neurotransmitters.[57-60]

Animal models used to study the NO pathway involvement in male urethral obstruction include guinea pigs, rabbits and fetal sheep. In 1997, Zhou reported that when the outlet was completely obstructed, there was upregulation of NADPH-related activity in the intramural ganglia of the guinea pig bladder at 12 hours following obstruction.[61-63] At 24 hours and 48 hours of complete obstruction there were increased levels of ganglionic cell death, with a 32% reduction in the number of neurons staining for NO activity at 48 hours. The authors hypothesized that NO may be involved in neuronal death in the bladder wall when the outlet is completely obstructed.

In the fetal sheep model, Levin et al showed that nNOS is maintained after partial outlet obstruction of 3-5 days duration.[64] Lieb et al reported that blood flow to the bladder following urinary drainage was regulated by nitric oxide.[65]

Since NO has been shown in both animal and human studies to mediate urethral relaxation, and it has also been shown that urethral relaxation can be inhibited by NO blockers and enhanced with NO donors, researchers have proposed the use of the NO donors in patients with functional obstruction of the LUT. Mamas et al[66] hypothesized that NO donors such as glyceryl trinitrate or isosorbide mononitrate could be used for either short term or long term urethral relaxation in spinal cord injured males with detrusor sphincter dyssynergia (DSD). While still unproven, such concepts of medical management for this condition would be welcome. Current treatment for DSD remains essentially surgical, with significant complications.

All animal studies of the role of NO in urethral obstruction have used male animals. The effects in the female animal model remain to be elucidated.

The role of NO in bladder overactivity and detrusor instability

The role of NO in bladder smooth muscle activity is not clearly understood. Initially it was thought that NO directly mediated detrusor smooth muscle relaxation. However, the degree of this effect *in vivo* was found to be less than initially anticipated[42,67-69] and has been described by some authors as having a "limited direct impact".[70] The factors refuting the importance of NO as a direct mediator of bladder relaxation were summarized by Haab in 2000[70] as: 1) a lack of NO release upon bladder distension, and 2) the limited effect on bladder function of agents that modulate cGMP formation. Most authors now describe NO as being an indirect mediator at the level of the bladder through modulation of bladder afferent neurotransmission[71] and interaction with other existing neurotransmitters in the bladder wall,[17] or through a combination of effects on both the bladder and the urethra. Despite uncertainty about the precise mechanism, there appears to be little doubt that NO plays a role in normal bladder detrusor function.

Experimental evidence for the role of NO in detrusor relaxation has come from animal studies. Inhibition of NO resulted in bladder overactivity and decreased bladder capacity in the unanesthetized rat[2] and the fetal

lamb.[32] In the latter, there was also increased residual volume and the authors hypothesized that this may have been due to failure of the outlet to relax appropriately. Haab[70] hypothesized that NO may regulate the threshold for bladder afferent firing and that this is an alternative explanation for the findings. Therefore, it may be misleading to consider the effects of NO on the bladder and urethra alone if it actually acts in the coordination of the micturition cycle Figure 1.

The smooth muscle relaxation effects of NO are mediated through cGMP-dependent protein kinase I (cGKI). Selective genetic knockout of cGKI is possible in mice and the effect on urinary tract function was reported by Persson et al.[72] While no gross changes in bladder weight or morphology were evident, the mice did display impaired urethral relaxation in response to NO donors. Functionally, mice that had cGKI knockout had increased frequency of urination, non-voiding bladder contractions, and a failure of NO-mediated urethral relaxation with voiding. This model suggests that there may be genetic determinants of signaling in the NO pathway that are responsible for "idiopathic" detrusor instability.

Interstitial cystitis

Clinically, interstitial cystitis (IC) is characterized by urinary frequency, urgency, and painful voiding. The role of NOS (both nNOS and iNOS) in the etiology and subsequent voiding dysfunction associated with IC has recently come under investigation. In 1996, Smith et al[73] described decreased levels of both urinary NO and the substrate cGMP in female patients with IC, implicating the NO pathway in the symptoms and the immunologic response of patients with IC. In 1997, it was demonstrated that, when injected in the rat bladder DMSO, which is given to patients with IC to provide relief of IC symptoms, directly released NO from both dissociated dorsal root ganglia and isolated strips of urinary bladder.[74] Other evidence that NO may be important in IC includes:

- IC is characterized by increased smooth muscle contraction, and NO promotes smooth muscle relaxation
- NOS inhibitors are associated with mast cell degranulation and inflammatory reactions[75]
- in animal models of bowel ulcers, increasing levels of substrates for NO improved ulcer healing[76,77]

Pilot studies using oral L-arginine supplementation in an open label, non randomized format suggested significant symptomatic improvement.[78,79] Korting et al in 1999[80] reported a double blind, randomized controlled trial of a single dose of 1.5 grams L-arginine per day. No statistical benefit from the supplementation of L-arginine was found in this study, but the authors suggested that a cross over study may have shown an effect.

In March 2000, a double blind, randomized cross over study was reported by Cartledge et al[54] who studied a group of 16 patients with IC and decreased urinary levels of NO. Patients were given L-arginine orally, 2.4 g per day, in an attempt to increase levels of the substrate for NOS to metabolize. There was a statistically significant improvement in symptom indices, but no significant decrease in urinary frequency measures or increase in voided volume.

Infectious and chemical-induced cystitis

The NO pathway has been implicated in the symptoms of urinary frequency, urgency and pain associated with infectious cystitis and in the chemical cystitis induced by cyclophosphamide (CYP). Since 1996, several authors including Kakizaki,[68] Vizzard[81] and Yoshimura,[82] have demonstrated that bladder inflammation upregulates the expression of NOS in bladder afferent neurons and that NO produced in bladder afferent pathways might be involved in the regulation of the voiding reflex following cystitis. Poljakovic[83] demonstrated that, in rats, *Escherichia coli* induces expression of iNOS in the urinary tract at the level of the bladder and kidney.

In 1999, Ozawa et al[84] topically applied NO donors intravesically and assessed subsequent bladder function in rats exposed to CYP. They found that topical NO donors acutely suppressed CYP induced bladder hyperactivity. This was described as "likely due to suppressed bladder afferent nerve activity, rather than direct detrusor smooth muscle relaxation". They hypothesized that NO donors could be useful in the treatment of the irritative symptoms associated with cystitis. Xu et al[85] found that treatment with a NOS inhibitor prior to CYP administration ameliorated the CYP-induced cystitis. They hypothesized that CYP induces increased expression of iNOS in bladder smooth muscle cells. This concept was further supported by Alfieri et al[86] who determined that, in rats, iNOS inhibitors (S-methylthiourea (MITU)), but not nNOS inhibitors, significantly reduced the effects of CYP on the bladder.

The NO pathway has also been implicated in the action of bacillus Calmette-Guerin (BCG). Oh et al[87] demonstrated that BCG upregulates gene and protein expression of iNOS and eNOS in normal rat bladders.

An important potential role for NO in the bladder of humans was demonstrated by Wall et al.[88] Thirty-seven

adults with indwelling Foley catheters for eight or more years contributed bladder biopsies. In addition to inflammatory infiltrates found in 100% of cases, 20 patients had squamous metaplasia, three had epithelial dysplasia and one had bladder carcinoma. iNOS macrophages were localized to the lamina propria. Bladder biopsies from cadaveric organ donors served as controls and iNOS was not detected in any of the samples. The authors concluded that iNOS expressed in macrophages in the bladders of patients with chronically indwelling catheters may lead to carcinoma of the bladder through sustained production of NOS products, particularly nitrosamines.

The role of NO in LUTS associated with female menopause

NOS levels in other organ systems, such as the cardiovascular system, can be regulated by administration of estrogen and estrogen receptor modulators such as Raloxifene.[89] Other researchers have reported that exogenous estrogen results in upregulation of eNOS in human cardiovascular tissue cultures.[90] Like NOS, estrogen receptors are found in abundance in the bladder, the urethra and the pelvic floor of women. In the lower urinary tract, estrogen treatment increases tissue mass and blood flow in the urethra and bladder[91] and increases alpha adrenergic receptors in the urethra.[92] Upregulation of NOS by estrogen has been demonstrated in the ovariectomised guinea pig bladder[93] and the ovariectomised rat urethra.[94]

Fluctuations in levels of the sex hormones estrogen and progesterone that occur during menstrual cycling and at the time of menopause are recognized to be associated with LUTS in women and to alter urodynamic parameters: 40% of non pregnant, premenopausal women report changing LUTS just prior to menstruation;[95] and the prevalence of detrusor instability detected on filling cystometry increases with time from the last menstrual period.[96] During pregnancy, LUTS are common and researchers have determined that the frequency of LUTS is not due solely to changes in urinary output and uterine pressure.[97-99] Later in life, lowering levels of estrogen during menopause have been implicated in the development of irritative voiding symptoms and the onset of *de novo* urinary incontinence. Iosif and Bekassy[100] found that 49% of 2200 menopausal women reported LUTS; 70% of these reported *de novo* urinary incontinence temporally associated with their final menstrual period. Twenty percent of women who seek attention for symptoms related to menopause report severe urinary urgency.[101] The effectiveness of estrogen supplementation in the management of LUTS associated with menopause is highly controversial and has been the subject of numerous randomized controlled trials that report conflicting results.[96] Management of NOS levels may provide a more productive alternative avenue of treatment for this widespread problem.

The role of NO in bladder ischemia

Ischemia and ischemia-reperfusion injury to the bladder have been reported to cause LUTS, including urinary retention due to impaired detrusor contractility, and detrusor instability.[102] The effect of ischaemia on the bladder is of clinical interest not only because ischaemia is produced during common operations on the cardiovascular system, but also because peripheral vascular disease associated with aging can cause tissue ischaemia. Authors have hypothesized that bladder dysfunction following ischaemia may be due to: impaired delivery of oxygen and nutrients; increased concentrations of waste products; decreased levels of intracellular ATP; and increased intracellular Ca^{2+}.[103] They also hypothesize that the duration of ischaemia may be a significant factor.[104] In animal models, researchers have demonstrated that ischaemia increases NO release in the bladder but following reperfusion, NO returned to pre-ischaemia levels. iNOS was detected in the infiltrated neutrophils in the muscular and submucosal regions of the bladder in this study.[102] The authors hypothesized that NO from leukocytes may be involved in bladder cell damage and that the initial increase in NO may have come from constitutive NOS resulting from hypoxia-induced vasodilation.

At the level of the urethra, ischaemia has been found to decrease NOS activity and impair NO-mediated urethral relaxation in animals.[105] Functionally we would expect such a reduction to lead to increased urethral pressure and potentially obstructive voiding symptoms.

Conclusion

A decade ago it was first shown that normal erections were critically dependent on the function of the NO/Guanylate Cyclase/cGMP pathway, and furthermore, that erectile dysfunction could be effectively treated by stimulation of this pathway (i.e., sildenafil). Since then, this pathway has also become a major focus of investigation in much of the rest of the lower urinary tract. In short, undeniably, NOS has been found to

have a significant impact on lower urinary tract physiology/pathophysiology. However, to date, most studies of NOS in the bladder and urethra have been conducted in animal models, primarily males. Thus, evidence for intuitively obvious gender-based differences in both the pathophysiology and therapeutics of lower urinary tract disorders is still lacking. The next logical step is therefore a better evaluation of NOS in animal models of both genders. As a further prelude to a determination of their efficacy in clinical trials, it will be critical to control not only for gender, but also age and tissue-specificity (i.e., effects on bladder vs. urethral symptoms), and finally, to address the issue of sex hormone interactions in randomized controlled trials. Nonetheless, it would seem that manipulation of this pathway is likely to lead to an improved understanding, diagnosis and treatment of lower urinary tract diseases/disorders. □

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