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The effect of N^G-monomethyl-L-arginine on bladder function

Robert J. Theobald Jr. *

Department of Pharmacology, Kirksville College of Osteopathic Medicine, 800 West Jefferson Street, Kirksville, Kirksville, MO 63501, USA

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Abstract

Recent studies have demonstrated the presence of nitric oxide synthase (NO synthase) in lower urinary tract tissues, however, its role in the detrusor is unclear. The current study was designed to determine if NO synthase inhibition alters detrusor activities, including micturition volume threshold, and inhibition of pelvic nerve-evoked contractions by various stimuli. In naive, anesthetized adult cats, inhibition of pelvic nerve-evoked bladder contractions, induced by hypogastric nerve stimulation or the intraarterial administration of NA, ATP, adenosine, β,γ-methylene ATP and 2-methylthio ATP, was measured before and after inhibition of NO synthase. The micturition volume threshold was also measured before and after NO synthase inhibition. L-NMMA decreased the micturition volume threshold by 38% (2 mg intravesical administration) or 80% (4 mg/kg i.a.). The magnitude of the micturition contractions was modestly increased. These results, and information in the literature, indicate that NO may play a role in the collection phase of the bladder cycle and any alteration of nitric oxide availability could induce or allow development of various bladder malfunctions, such as small bladder diseases, like interstitial cystitis.

Keywords: Purinergic; Micturition volume threshold; Inhibition; Nitric oxide (NO)

1. Introduction

Recent studies have demonstrated the presence of nitric oxide synthase (NO synthase) in tissues of the lower urinary tract, including detrusor, trigone and urethral smooth muscle (Triguero et al., 1993; McNeill et al., 1992). With these studies, investigation of the role of nitric oxide (NO) in lower urinary tract function is continuing. Several studies have demonstrated a role of NO as a mediator of urethral smooth muscle relaxation in animals (Andersson et al., 1992; Thornbury et al., 1992; Persson et al., 1992). Other studies suggested the presence of, and a possible role for, NO in human tissues, including detrusor (James et al., 1993; Smet et al., 1994) and trigone (Smet et al., 1994). The role, if any exists, of NO in detrusor smooth muscle is unclear.

Other studies have indicated an effect of NO synthase inhibitors on neural components of the lower urinary tract, both parasympathetic and sympathetic. NO synthase inhibitors have been shown to alter the release of catecholamines by hypogastric nerve stimulation (Thatikunta et al., 1993). These authors demonstrated that inhibition of NO synthase by L-N^G-nitro-arginine (L-NNA) produces a reduction of sympathetic nerve release of noradrenaline and adrenaline (A) in the anal sphincter of the opossum. Hypogastric nerve stimulation produces an inhibition of pelvic nerve-evoked bladder contractions and it is part of the neural pathway mediating feedback inhibition of the bladder in the cat (De Groat and Theobald, 1976). Therefore, inhibition of NO synthase may also alter detrusor function by changing the activity of this feedback, either basal activity or evoked activity. Another study (Wiklund et al., 1993) suggests that endogenous NO may be involved in both pre- and post-junctional modulation of cholinergic transmission. In this study, the investigators demonstrated that NO synthase inhibition enhanced electrically induced contractions, while exogenously administered NO reduced tritiated choline overflow and contractile activity induced by exogenously administered agents.

The current study was designed to determine if NO synthase inhibition altered various detrusor parameters, including micturition volume threshold, and inhibition of pelvic nerve-evoked contractions by hypogastric nerve stimulation and the exogenous administration of certain agents.

* Corresponding author. Tel.: +1 816 626 2316; fax: +1 816 626 2728.

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2. Materials and methods

Naive, adult cats of either sex, anesthetized with sodium pentobarbital, 35 mg/kg i.p., were used in an acute preparation. The lower urinary tract was exposed via a midline abdominal incision. The bladder was cannulated through an incision in the internal urethra approximately 4–5 cm distal to the neck of the bladder with a cannula, 2 mm i.d. The cannula was filled with physiological saline and connected to a Statham pressure transducer for the continuous monitoring of intravesical pressure and as a route for instilling fluid in the bladder. In 19 experiments that determined the effects of the NO synthase inhibitor, N^G-monomethyl-L-arginine (L-NMMA), on nerve- and drug-induced responses, the pelvic and hypogastric nerves were isolated, sectioned bilaterally and prepared for electrical stimulation. In 30 experiments designed to determine micturition volume thresholds, the nerves were not sectioned, keeping the neuraxis intact. The left radial vein and the right femoral artery were cannulated for the administration of drugs and monitoring arterial pressure respectively. A cannula was passed through the left renal artery into the abdominal aorta for the intraarterial administration of drugs. The left internal iliac artery was ligated to maximize drug delivery to the bladder and the right ureter was ligated to allow control of bladder volume.

During the experiments determining micturition volume thresholds, fluid was instilled into the bladder through the cannula in the internal urethra at a rate of 0.018 ml/kg/min. This rate was chosen using information from a study performed by Klevmark (1974) in which he determined that varying infusion rates altered responses of detrusor and that this rate, 0.018 ml/kg/min, was physiological for cats. Fluid was instilled at this rate using a Harvard Infusion/Withdrawal pump until a large micturition contraction occurred. After the micturition contraction, the bladder was gently emptied, the fluid measured and this procedure was repeated. Again the bladder was emptied, then 2 mg of the NO synthase inhibitor, dissolved in a minimal volume of saline, was instilled into the bladder lumen via the catheter, left in the bladder for 15 min after which the inhibitor was withdrawn and the bladder gently flushed with saline. Again, fluid was instilled at the above rate until a large micturition contraction occurred. In some experiments, the NO synthase inhibitor was administered intraarterially at a dose of 4 mg/kg. In most experiments, 15 min elapsed before a micturition contraction was induced with fluid instillation. In some experiments, L-arginine (L-Arg) was administered, either 20 mg (instilled intravesically) or 40 mg/kg (i.a.), to reverse the effects of the NO synthase inhibitor.

In experiments designed to study the effects of NO synthase inhibitors on nerve- and drug-induced responses in the bladder, bladder contractions were evoked with trains of pelvic nerve stimulation at 5 Hz, 0.05 ms duration at optimum intensity. The contractions were evoked every 30 s and inhibition of the contractions was induced by hypogastric nerve stimulation, at 15 Hz, 0.05 ms duration at various intensities, or exogenous administration of noradrenaline, ATP, β,γ-methylene ATP, adenosine and 2-methythio ATP administered via the renal artery cannula.

Noradrenaline, ATP, β,γ-methylene ATP, adenosine, 2-methythio ATP and L-Arg were purchased from Sigma (St. Louis, MO, USA). N^G-monomethyl-L-Arg was purchased from Research Biochemicals International (RBI; Natick, MA, USA). All drugs were dissolved in normal saline prior to administration.

Responses were recorded on a Grass polygraph. Statistical significance was determined using Student's t-test to compare individual means of each group. In all instances, $P < 0.05$ was considered significant.

3. Results

3.1. Effect of L-NMMA on adrenergic- and purinergic-induced inhibition of pelvic nerve-evoked bladder contractions

L-NMMA did not significantly alter inhibition of pelvic nerve-evoked bladder contractions when administered intraarterially (4 mg/kg i.a.; not shown) or instilled intravesically (2 mg; Fig. 1). The inhibition produced by hypogastric nerve stimulation and exogenous administration of noradrenaline was not changed significantly, however, at the higher intensities or doses, there was a tendency towards decreased inhibition. In 75% of the experiments, the inhibition produced by hypogastric nerve stimulation at 30 V was decreased, while in only 33% of the experiments was the noradrenaline inhibition decreased. Fig. 2 shows the effect of intravesical administration of L-NMMA (2

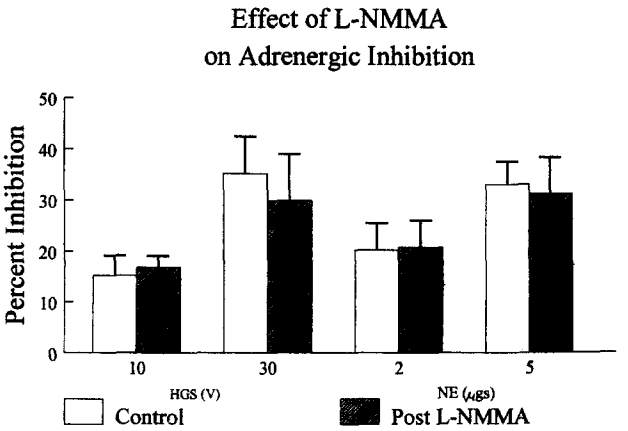

Fig. 1. Effect of L-NMMA on inhibition of pelvic nerve-induced bladder contractions elicited by hypogastric nerve stimulation (HGS; 10 and 30 V, 15 Hz, 0.05 ms) and exogenous administration of noradrenaline (NA; 2 and 5 μg i.a.). Each bar represents the mean ± S.E.M. of 3–19 experiments.

mg) on purinergic inhibition of pelvic nerve-evoked bladder contractions. As seen in the top panel, neither the inhibition of bladder contractions by ATP nor by adenosine was altered significantly. The inhibition of bladder contractions by β,γ-methylene ATP, shown in the middle panel, was not altered significantly, nor was the inhibition of bladder contractions by 2-methythio ATP, shown in the bottom panel. The evidence suggests that NO may not be involved in adrenergic or purinergic mediated inhibition of pelvic nerve bladder contractions produced by hypogastric nerve stimulation or exogenous administration of drugs.

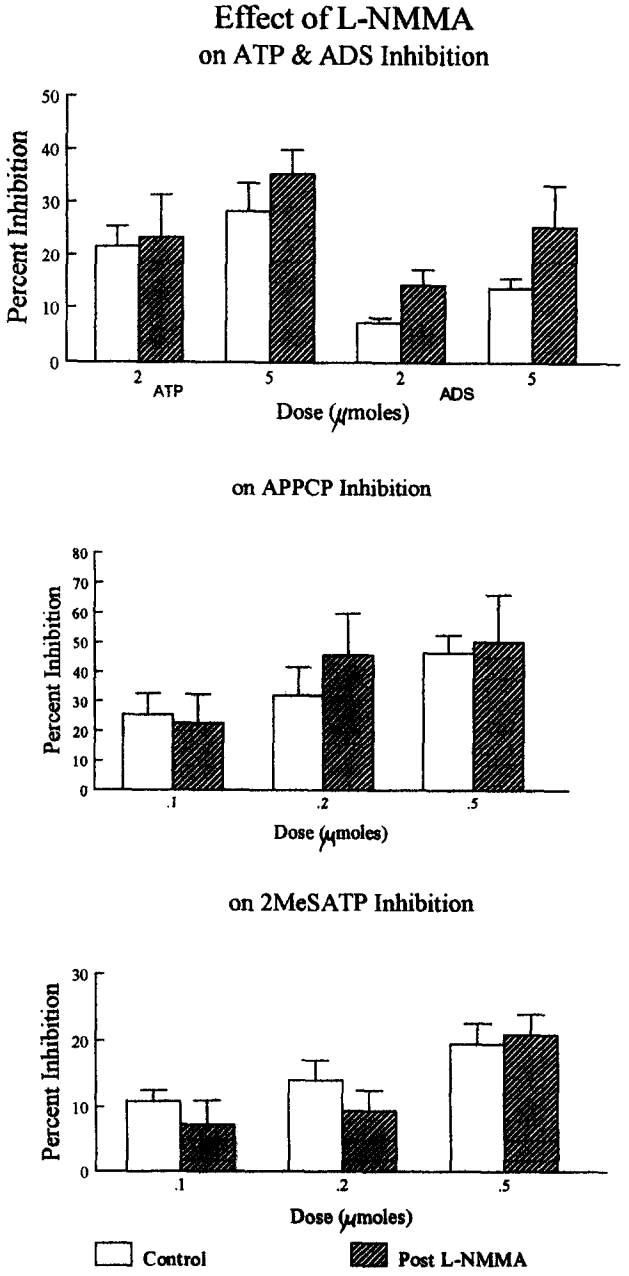

Fig. 2. Effect of L-NMMA on inhibition of pelvic nerve-induced bladder contractions elicited by the intraarterial, exogenous administration of ATP (2 and 5 μmol), adenosine (ADS; 2 and 5 μmol) (top panel), β,γ-methylene ATP (APPCP; 0.1–0.5 μmol) (middle panel) and 2-methylthio ATP (2MeSATP; 0.1–0.5 μmol) (bottom panel). Each bar represents the mean ± S.E.M. of 3–19 experiments.

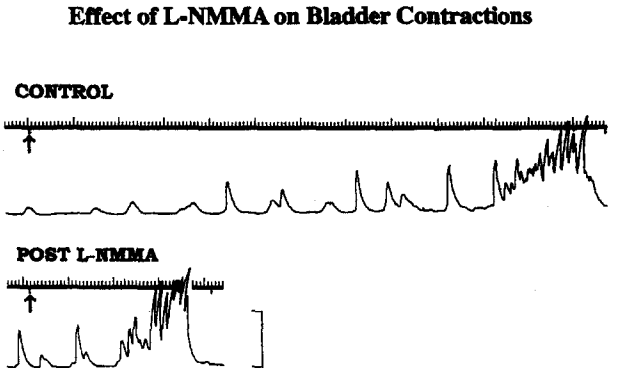

Fig. 3. Effect of L-NMMA (4 mg/kg i.a.) administration on the micturition volume threshold. The arrows indicate the point at which the infusion (0.018 ml/kg/min) was started. Time is indicated by the tic marks at the top of each tracing. Each mark equals 5 s. Vertical calibration equals 40 cm of H_2O.

3.2. Effect of L-NMMA on micturition volume threshold

The effects of L-NMMA (4 mg/kg i.a.) on micturition volume threshold is shown in Fig. 3. As seen in the figure, the volume of saline, slowly infused into the bladder, required to evoke centrally mediated micturition contractions, the micturition volume threshold, was decreased by L-NMMA. Although the magnitude of the contractions produced was not changed significantly, L-NMMA increased this magnitude in 65% of the experiments (not shown). Fig. 4 summarizes these experiments. As shown in Fig. 4, intravesical instillation of L-NMMA, 2 mg, significantly reduced the volume required to evoke these centrally mediated contractions from a mean volume of 7.8 to 4.4 ml. Intraarterial administration of L-NMMA, 4 mg/kg, reduced the volume required to evoke these centrally mediated contractions from a mean volume of 11 to 2.16 ml. The reduction of micturition volume threshold produced by intraarterial administration of L-NMMA was greater (80%) than the reduction produced by the intravesical administration (38%), although the difference was not statistically significant. Experiments using a lower dose of L-NMMA (1 mg i.a.) produced less reduction (mean 18%) of micturition volume threshold. This suggests that the reduction of micturition volume threshold by L-NMMA may be a dose-related phenomenon.

3.3. Effect of L-Arg, after L-NMMA, on micturition volume threshold

In several experiments (8), L-Arg was administrated in an attempt to reverse the effects of L-NMMA on micturition volume threshold. As can be seen in Fig. 5, L-NMMA decreased the micturition volume threshold when administrated either i.a. (top panel) or intravesically instilled (bot-

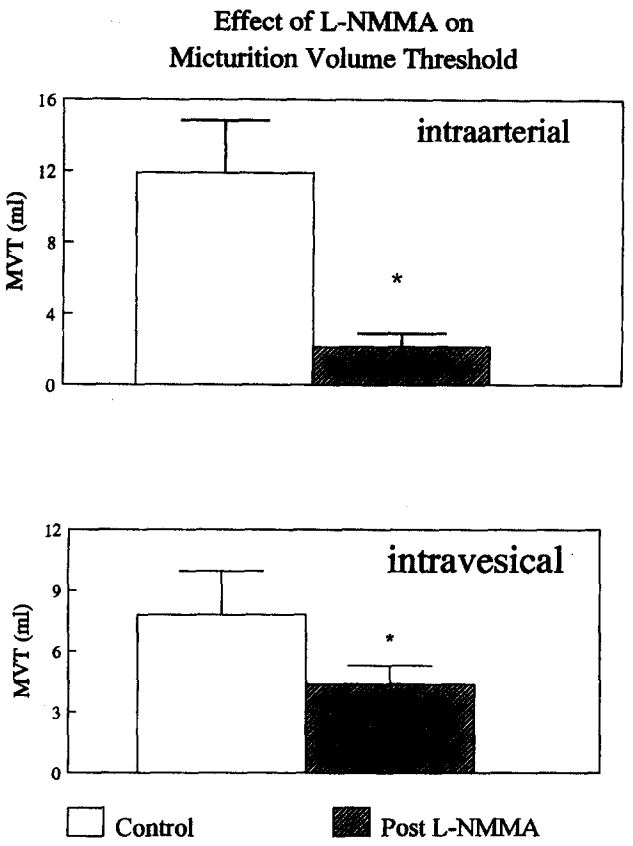

Fig. 4. Effect of L-NMMA instilled (2 mg; bottom panel) intravesically or administered (4 mg/kg i.a.; top panel) intraarterially on the volume required to elicit a micturition contraction. Each figure represents the mean ± S.E.M. of 3–19 experiments. * $P < 0.05$.

tom panel). The differences in the values in Fig. 4 and Fig. 5 are due to the fact that the control and L-NMMA values in Fig. 5 represent the data only from the animals that subsequently received L-Arg. As seen in Fig. 5, L-Arg did not significantly reverse the effects of L-NMMA on micturition volume threshold, however, the micturition volume threshold was increased in all experiments in which L-Arg was administered except 1.

4. Discussion

The role of NO in lower urinary tract function is unclear. The presence of NO synthase has been demonstrated in numerous tissues of the lower urinary function, including detrusor smooth muscle, urothelium, trigonal smooth muscle and the urethra. Most evidence to date suggests that NO helps mediate relaxation of the closure tissue, including the trigone and urethra, allowing urine flow to be initiated and maintained during the expulsion phase of the urinary tract cycle. There was nothing to suggest a role for NO, an agent that relaxes smooth muscle, in the detrusor. However, the presence of NO synthase in detrusor smooth muscle indicated a possible role, perhaps as a mediator of adrenergic or purinergic inhibition of bladder contractions.

Various agents, including noradrenaline and some purines, relax detrusor smooth muscle and inhibit pelvic nerve-evoked bladder contractions (Theobald and De Groat, 1989). Noradrenaline and ATP, released by hypogastric nerve stimulation produce these responses. The possibility that NO mediated one or both of these responses was tested. The current data demonstrates that NO synthase inhibition does not significantly alter the inhibition of detrusor activity induced by either adrenoceptor or purinoceptor agonists, suggesting that NO is not a mediator of adrenergic or purinergic inhibition.

Another possible role for NO in detrusor could be as a mediator of bladder relaxation during the collection phase of the urinary tract cycle. The decrease in micturition volume threshold produced by L-NMMA suggests that this

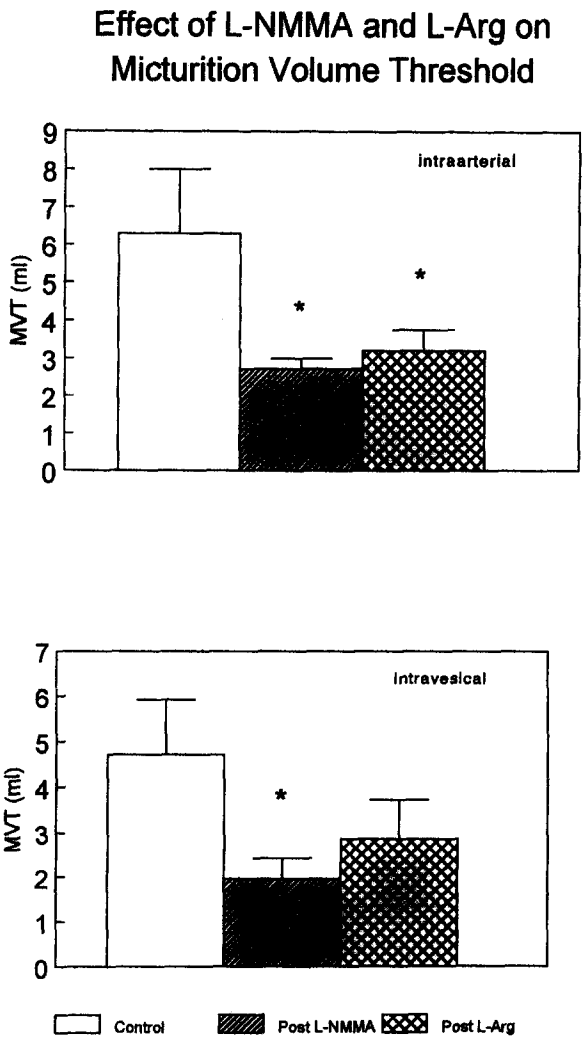

Fig. 5. Effect of L-NMMA and L-Arg on micturition volume threshold. Intravesical instillation of L-NMMA (2 mg) and L-Arg (20 mg) is shown in the bottom panel, while intraarterial administration of L-NMMA (4 mg/kg) and L-Arg (40 mg/kg) is shown in the top panel. Each figure represents the mean ± S.E.M. of 3–8 experiments. * $P < 0.05$.

NO synthase inhibitor may be producing an effect in cats similar to that reported in the anal sphincter of the opossum (Thatikunta et al., 1993). These investigators reported that L-NMMA decreased the release of noradrenaline elicited by hypogastric nerve stimulation. A decrease of noradrenaline released by hypogastric nerve stimulation would produce a decrease in micturition volume threshold because noradrenaline is the inhibitory neurotransmitter that mediates inhibition of bladder activity in the negative feedback pathway of the bladder. This pathway allows the bladder to collect large urine volumes at low intravesical pressures (De Groat and Theobald, 1976; Kuru, 1965). Another factor that may be involved in these results is that L-NMMA may be interrupting endogenous NO actions on cholinergic transmission, either by indirectly increasing acetylcholine output in response to nerve transmission, or by altering the post-synaptic effects of endogenous NO. These actions of NO on cholinergic transmission have been demonstrated in other tissues, such as longitudinal muscle myenteric plexus preparations of guinea-pig isolated ileum (Wiklund et al., 1993). In addition, recent evidence suggests a possible role for NO in sensory activity (Uvelius et al., 1994) based on the presence of NO synthase-immunoreactive cell bodies present in dorsal root ganglia (DRG) of the thoracic and lumbar regions of the spinal cord (Vizzard et al., 1994). NO could have some role in the negative feedback system of the bladder that is interrupted by NO synthase inhibitors, thereby decreasing the micturition volume threshold as seen in the current experiments. Finally, NO could be acting directly on detrusor smooth muscle to produce relaxation. Numerous studies have shown that NO relaxes several types of smooth muscle (Persson and Andersson, 1992; Andersson et al., 1992), including bladder smooth muscle (James et al., 1993).

The response of the system to intravesical instillation of NO synthase inhibitors was not significantly different than the response to intraarterial administration. Micturition volume threshold decreased after intravesical instillation of L-NMMA by 38% while micturition volume threshold decreased 80% after intraarterial administration. Although not marked, the difference in these responses may be due to the localization of NO synthase. Several recent papers used nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase histochemistry and NO synthase immunochemistry to localize NO synthase in the lower urinary tract (Triguero et al., 1993; McNeill et al., 1992; Alm et al., 1995). NADPH-diaphorase-positive nerve fibers are present in bladder wall and adjacent small ganglia, the adventitial and muscular layers adjacent to the urothelium of the rat (McNeill et al., 1992). These authors also noted NADPH-diaphorase-positive perikarya in the major pelvic ganglia and the T_{13}-L_2, L_6 and S_1 dorsal root ganglia, but not in the perikarya of the inferior mesenteric ganglia. NADPH-diaphorase reactivity was shown to be coincident with strong NO synthase immunoreactivity in the major pelvic ganglia of the rat (Alm et al., 1995), supporting this earlier study. NADPH-diaphorase-positive fibers were also noted in sheep trigone and urethra, but not detrusor or ureters (Triguero et al., 1993). The presence of NO synthase, demonstrated by NADPH-diaphorase histochemistry, in various bladder tissues suggests that NO synthase inhibitors could be effective when presented to the serosal side of the tissue, that is, administered via the circulation rather than intravesically instilled in the bladder. Perhaps, the urothelium delays or disrupts the ability of the inhibitor agents to reach the site of NO synthase when they are instilled intravesically.

The presence of NO synthase in the muscular layer of the bladder and in the dorsal root ganglia strongly suggests a role for NO in bladder function. The ability of NO synthase inhibitors to decrease release of noradrenaline from sympathetic nerves, the ability of NO to relax smooth muscle, and the decrease in micturition volume threshold seen after the intraarterial administration of these inhibitors provides evidence for a possible role for NO. Perhaps, NO has a neuromodulatory/neurotransmitter role during the collection phase of the bladder cycle. As the bladder is filling, the negative feedback pathway increases activity and produces a release of noradrenaline from the hypogastric nerves that decrease pelvic ganglionic transmission and help relax bladder smooth muscle. The ability of L-NMMA to decrease noradrenaline release (Thatikunta et al., 1993) suggests an interaction between noradrenaline and NO and that any disruption of the production or function of NO may alter the ability of the bladder to collect and store urine. A decrease in bladder capacity and compliance could occur leading to an increase in voiding frequency. Detrusor contraction activity would be increased, perhaps to the point of producing painful contractions, or a type of cramping. Symptom presentation could be similar to those seen with certain bladder diseases, such as interstitial cystitis, hyperreflexia or dysnergia. Clearly, NO, and the inhibition of NO synthase, produces changes in bladder function.

Support for a role for NO involvement in relaxation of the detrusor during the collection phase of micturition, perhaps released from the urothelium, can be seen in a recent paper by Levin and coworkers (Levin et al., 1995). The authors demonstrated that removal of the urothelium resulted in an increase in the contractile response of cat bladder strips to various stimuli, including field stimulation. They concluded that the mucosa has an inhibitory influence on the bladder. Evidence for the presence of NO synthase in urothelium is somewhat clouded. Several studies have noted that NADPH-diaphorase-positive neuronal structures are present in proximity to the urothelium in humans (Smet et al., 1994), pig (Persson et al., 1992) and rat (McNeill et al., 1992). However, in other studies, there was a lack of NO synthase-immunoreactive responses in the urothelial tissue in pig (Persson et al., 1992) and rat (Alm et al., 1993). This contrasting information may be

due to several factors. There may be some relaxing factor other than NO present in urothelium that is removed whenever the urothelium is stripped from the muscle tissue, allowing increased contractile activity as seen in the study by Levin and coworkers (Levin et al., 1995). There could also be some form of NO synthase that does not react to the immunoreactive probe used in the earlier studies. This was suggested earlier by Andersson (Andersson, 1993). Clearly, more studies must be done to determine if NO is released by urothelium, or if there is another relaxing factor present in the urothelium.

The effect of L-Arg in reversing the effect of L-NMMA was not complete, as seen in Fig. 5. Although the reason for this is undetermined, Thatikunta et al. (1993) obtained similar results in their study where the effects of L-NNA were not completely reversed by L-Arg. They offered no explanation, however, there are several factors that could influence this lack of reversal. The dose of L-Arg administered to reverse the effects of L-NMMA may not have been sufficient. The animals may distribute or metabolize L-Arg rapidly, not allowing the amount administered in a bolus to have a significant effect.

In summary, the data presented here indicate that NO may not be involved in adrenergic or purinergic inhibition of pelvic nerve-evoked bladder contractions; NO does appear to have a role in relaxation of detrusor smooth muscle during the collection phase. NO's role during collection could be in the sensory input from the bladder to the spinal cord, in activation mechanisms for micturition at the spinal level or in the periphery, or perhaps in combination at all these sites. Subsequently, anything that alters or disrupts NO availability could alter the bladder's ability to collect and store urine at low pressure, leading to significant symptoms. Investigations need to continue to focus on the possible role of NO in bladder function, particularly as a mediator of detrusor relaxation during urine collection.

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