Effects of L-arginine and L-NAME on chronic partial bladder outlet obstruction in rabbit

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L-arginine and L-NAME on chronic partial bladder outlet obstruction in rabbit. Am J Physiol Regul Integr Comp Physiol 293: R2390–R2399, 2007. First published October 10, 2007; doi:10.1152/ajpregu.00508.2007.—Nitric oxide (NO), a neurotransmitter responsible for relaxation activity in the lower urinary tract and corpus cavernosum, is synthesized from L-arginine in a reaction catalyzed by NO synthase (NOS). Nitric oxide (NO) is synthesized from L-arginine by nitric oxide synthase (NOS). NOS can be inhibited by Nω-nitro-L-arginine methyl ester (L-NAME) and stimulated by supplementing the diet with L-arginine. The aim of this study was to investigate the influence of NOS activity on the response of rabbits to chronic partial bladder outlet obstruction (PBOO). Surgical PBOOs (2 and 8 wk) were performed on male New Zealand White rabbits. Before obstruction, one-third of the animals were premedicated for 7 days with L-NAME and another third with L-arginine. The results are summarized as follows. First, bladder weight after 8-wk PBOO was significantly lower in animals treated with L-arginine compared with both untreated and rabbits treated with L-NAME. Second, contractile function decreased progressively with PBOO duration. However, after 8 wk of PBOO, the L-arginine group had significantly greater contractile function compared with the no-treatment group, and the L-NAME group had significantly lower contractile function compared with the no-treatment group. Third, at 8 wk following PBOO, the level of protein oxidation and nitration was lowest for the L-arginine group and highest in the L-NAME group. These studies clearly demonstrated that increasing blood flow by stimulating NOS significantly protected the bladder from PBOO dysfunctions, whereas inhibiting blood flow by L-NAME enhanced the dysfunctions mediated by PBOO.

NITRIC OXIDE (NO), a neurotransmitter responsible for relaxation activity in the lower urinary tract and corpus cavernosum, is synthesized from L-arginine in a reaction catalyzed by NO synthase (NOS) (6). NO is also known to be a powerful and ubiquitous regulator of vascular tone, and thus in several systems is shown to regulate blood flow. In cardiac studies, impaired release of NO may be an important factor in the development of ischemia-reperfusion (I/R) injury. Conversely, it has been demonstrated that administration of NO donors or L-arginine reduces infarct size and improves the postischemic recovery of cardiac performance and endothelial function in animal models of myocardial ischemia and reperfusion (28, 36, 37). Similarly, other studies have shown that exogenously administered L-arginine decreases oxidative stress in the liver and brain (9) and that an increase in NO formation has a beneficial effect on the progression of cellular and molecular parameters of tubulointerstitial fibrosis caused by obstruction of the ureter (27). With regards to bladder function, our previous study demonstrated that feeding rabbits a diet high in L-arginine was beneficial for both control rabbits and those with 2 wk of severe partial bladder outlet obstruction (PBOO) (38).

Contradicting the protective effects, NO is reported to generate free radicals in I/R injury, which has been shown in other studies to be one of the primary causes of progression of bladder dysfunction (3). It has been demonstrated that NO-related damage due to nitrotyrosine was elevated with PBOO (23). Pretreatment with Nω-nitro-L-arginine methyl ester (L-NAME), which acts as a competitive inhibitor of NOS, significantly inhibited the generation of nitrotyrosine (8). Also, reports by Saito et al. (30–32) showed that treatment with L-NAME can reduce apoptosis induced by I/R in the bladder and significantly increase the contractile responses compared with the I/R group without L-NAME.

Because of this conflicting evidence and the fact that most studies have utilized an acute model of PBOO, we chose to investigate the effects of administration of L-arginine and L-NAME on the response of rabbits subjective to acute and chronic PBOO.

MATERIALS AND METHODS

Experimental protocol. All methodologies were approved by the Institutional Animal Care and Use Committee of the Stratton Veterans Affairs Medical Center, Albany, New York. Thirty-six young New Zealand White male rabbits (3–5 kg; 15–20 wk old) were obtained from Millbrook Breeding Labs (Amherst, MA). Animals were divided into three major groups labeled as control, 2-wk obstruction, and 8-wk obstruction. Each major group was composed of three subgroups of four rabbits each. Subgroup 1 was on a control diet containing a normal L-arginine concentration (0.76% arginine) and had osmotic pump with water. Subgroup 2 was premedicated with L-NAME (dissolved in water) by implanting an osmotic pump in the subcutaneous tissue behind the rabbit’s neck. The pump was set at an infusion rate of 6 mg·kg⁻¹·day⁻¹ and allowed 1 wk for equilibrium to reach adequate serum levels of L-NAME. The infusion was continued during the entire experimental period. The pump was changed every 4 wk in the 8-wk obstruction group. Subgroup 3 was on a diet containing 7% L-arginine.

Surgical procedure for creating PBOO. Rabbits in 2-wk and 8-wk obstruction groups were anesthetized using 25 mg·kg⁻¹ ketamine [H18528] kg ketamine [H11002] /H11002 /H18528 /H11002 /H11002 day⁻¹ and allowed 1 wk for equilibrium to reach adequate serum levels of L-NAME. The infusion was continued during the entire experimental period. The pump was changed every 4 wk in the 8-wk obstruction group. Subgroup 3 was on a diet containing 7% L-arginine.

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mg·kg xylazine−1 given intramuscularly and each bladder was catheterized with an 8-French Foley catheter, and the bladder was exposed through a midline incision. Under isoflurane (1–3%) anesthesia, mild bladder outlet obstruction was created by placing a 2-0 silk ligature loosely around the catheterized urethra, and the catheter was removed. Surgical incision of the PBOO procedure and all materials were the same in all groups. The wound was closed in layers. Pain medication (buprenorphine, 0.1 mg/kg im, twice daily) and antibiotics (gentamicin, 4 mg/kg im, daily) were administered for the first 2 days postoperatively.

**Tissue preparation.** After 2 and 8 wk postoperative, animals from each group were euthanized with pentobarbital sodium. The bladder was harvested from each animal and placed in Tyrode’s solution (in mM: 124.9 sodium chloride, 2.5 potassium chloride, 23.8 sodium bicarbonate, 0.5 magnesium chloride, 0.4 sodium biphosphate, 1.8 calcium chloride, and 5.5 glucose). The perivascular fat and connective tissues were removed, and the bladders were weighed. Four full-thickness bladder strips were prepared from the bladder body for contractile studies. Two full-thickness strips were fixed in buffered formalin for histological analysis. The remainder of the bladder was separated into the mucosal and muscle layers and stored at formalin for histological analysis. The remainder of the bladder was cut into 2-mm segments and stored at −80°C.

**Histology.** Each fixed section of bladder was embedded in paraffin on edge to provide a cross-sectional view of the bladder after microtome sectioning. Five-micrometer-thick sections were cut from each block and mounted on positively charged slides, deparaffinized in xylene, and used for the various histological and immunohistochemical studies.

**Smooth muscle and collagen staining.** Five-micron-sections were stained with Masson’s Trichrome Kit (Richard Allan Scientific, Kalamazoo, MI). The blue-stained collagen and red-counterstained muscle were highlighted for each image by using Image Pro Plus (Media Cybernetics, Silver Springs, MD) image analysis software by manually selecting the pixel values of each, using the color cube-based tool in the count/size application.

**Immunostaining for vascular endothelium by using CD31.** Tissue sections were stained with the mouse monoclonal antibody anti-CD31, clone IC/70A (DAKO, Carpinteria, CA) on the ES automated immunostainer (Ventana Medical Systems, Tucson, AZ) using an avidin-biotin and diaminobenzidene chromagen detection kit followed by a light hematoxylin counterstain. Immunostaining primary antibody (100 μl) was applied for 32 min at a dilution of 1:20 in antibody diluent (DAKO). The secondary antibody in the kit was substituted with biotinylated goat anti-mouse predilute (Lab Vision). Negative controls without antibody were performed. After the immunostaining, all slides were dehydrated through graded alcohols and two changes of xylene and then mounted with Permound (Fisher Scientific) mounting medium.

**Neurofilament staining.** Denervation was estimated immunohistochemically using antibodies to neurofilaments (21) and biochemically by measuring neurotransmitter synthesis with an assay for choline acetyltransferase (ChAT) (19).

Nerve damage is investigated through immunohistochemical evaluation of the presence and density of neurofilaments within the axons of bladder nerves. Tissue sections were stained with the mouse monoclonal antibody antineurofilament clone 2F11 (Neomarkers, Fremont, CA) on the ES automated immunostainer (Ventana Medical Systems) using an avidin-biotin and diaminobenzidine chromagen detection followed by a light hematoxylin counterstain. Neurofilament primary antibody (100 μl) was applied for 32 min dilution of 1:20 in antibody diluent (DAKO). The secondary antibody in the kit was substituted with biotinylated goat anti-mouse predilute (Lab Vision). Negative controls without antibody were performed. After the immunostaining, all slides were dehydrated through graded alcohols and two changes of xylene and then mounted with Permound (Fisher Scientific) mounting medium.

**Nerve calculation.** The nerve data, including size and quantity were acquired from the stained cross sections and were done as described for the blood vessel density quantitation.

The transverse section of each specimen was captured in four frames at ×100 magnification. The nerves in each image were highlighted using the Image Pro Plus software by manually selecting the pixel value within the stained nerves using the color cube-based tool in the count/size application of the program. The area measurement was then selected for all the nerves highlighted, and the data were saved to Excel and later compiled and compared for all the groups. For control tissues, the mean diameter of the nerves was identified arbitrarily, making 50% large and 50% small nerves. The effects of L-NAME and L-arginine on nerve size used the mean diameter from the control tissues to determine the relative changes in the percentage of large and small nerves.

**Citrate synthase assay.** Citrate synthase (CS) is the rate-limiting enzyme of the TCA cycle, which provides substrate for the respiratory chain. A reduction in respiratory chain substrates would lead to decreased oxidative phosphorylation. Therefore, CS was the enzyme chosen to assess further mitochondrial oxidative metabolism.

CS activity was determined, as described previously (12). In brief, bladder tissue (20 mg/ml) is homogenized in cold 0.225 M mannitol and 0.075 M sucrose. An aliquot is mixed with oxaloacetate, acetyl-CoA, DTNB, and Triton X-100. The free coenzyme A generated by CS activity reacts with DTNB to form a colored compound that is quantified at 412 nm by using a spectrophotometer (model 2001;
Hitachi Instrument, Danbury, CT). Quantitation of total protein was performed by using the Micro BCA protein assay reagent kit (Pierce, Rockford, IL).

Choline acetyltransferase. The method was adapted from the procedure of Fonnum (11). For each supernate, 100 and 200 μl aliquots were incubated in a water bath at 37°C for 10 and 20 min in 20 ml scintillation vials with 200 μl of a reaction mixture consisting of 9.85 mM acetyl-CoA, 0.2 mM 3H-acetyl-CoA (200 mCi/mmol), 8 mM choline, 50 mM sodium phosphate, 0.3 M sodium chloride, 20 mM EDTA, and 96 nM physostigmine. After the incubation time had elapsed, the solution was diluted with 5 ml (0.1 M) sodium phosphate, and the reaction was stopped with 2 ml of acetonitrile containing 5 mg/ml tetraphenylboron.

After the reaction was stopped, 10 ml of Betamax scintillation fluid were added slowly to each scintillation vial. The vial was then shaken lightly. The samples were allowed to stand for 1 h while the phases were separated, thus extracting the acetylcholine into the toluene phase, while the radioactive acetyl-CoA stayed in the aqueous phase. The activity in the vials was counted in a Packard scintillation counter.

The counts per minute were converted to disintegrations per minute by dividing the counts per minute by the efficiency of the counter. The ChAT activity was calculated from the disintegrations per minute utilizing the specific activity and the tissue concentration of the homogenate. Quantitation of total protein was performed by using the Micro BCA protein assay reagent kit (Pierce, Rockford, IL). The ChAT activity is given in picomoles of acetylcholine generated per minute per gram of tissue.

SDS-PAGE and Western blot analysis. Oxidatively modified proteins have been used as early markers of oxidative stress in a variety of physiological and pathophysiological processes. Protein carbonylation is the most widely used biomarker for oxidative damage to proteins and reflects cellular damage induced by multiple forms of reactive oxygen species (ROS) (23a). Nitrotyrosine, demonstrated to be a marker of free radical damage due to reactive nitrogen species (RNS), has been shown to be significantly elevated with PBOO (23, 26).

Carbonylation and nitrotyrosination of proteins were determined in a similar manner except for difference in primary antibody and buffers. Extraction of proteins and Western blot analysis were done according to the method previously described (8). For carbonyl content, the conversion of carbonyl to dinitrophenyl groups was performed by washing the membranes in 24 ml of 2N HCl for 5 min. Then 1 ml of 2.5 mg/ml dinitrophenyl hydrazine (DNP) was added and allowed to wash for another 5 min. The membranes were then blocked with Blotto (5% wt/vol nonfat dry milk) in Tween-20 Tris buffered saline solution (TTBS [0.05% vol/vol Tween 20, 10 mM Tris, pH 7.5, 100 mM NaCl]) for 45 min. Membranes were probed with primary antibody, which was goat anti-DNP (Bethyl Laborato-

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**Fig. 2.** A: maximal contractile responses to field stimulations (2 Hz, 8 Hz, and 32 Hz). B: maximal contractile response to ATP, carbachol, and KCl. Each bar is the mean ± SE of 4 individual rabbits. There are 16 bladder strips used for each bar. *Significantly different from control (P < 0.05); **significantly different from no-treatment group (P < 0.05). C, control; O2W, 2-wk obstruction; O8W, 8-wk obstruction.
KCl, with the L-arginine being relatively partially preserved similar in the contractile responses to ATP, carbachol, and than no treatment after 8 wk of PBOO. This pattern was quite (B). However, denudation of mucosa and atrophy of smooth muscle cell were characteristic in both no-treatment and L-NAME groups of 8-wk obstruction.

The magnitude of this increase was significantly less for the increase in bladder weight in all three groups, although the increase in L-arginine group (Fig. 1). However, rabbits treated with L-arginine had signif-

cantly greater contractile function compared with no treat-

ment, and L-NAME treatment had lower contractile function than no treatment after 8 wk of PBOO. This pattern was quite similar in the contractile responses to ATP, carbachol, and KCl, with the L-arginine being relatively partially preserved and, in this case, the L-NAME group having lower contractile responses (Fig. 2B).

The histology of the bladder wall is similar among all control subgroups (Fig. 3A). Morphological changes after 2-wk obstruction all showed hyperplasia and hypertrophy of mucosa and smooth muscle cell among the no-treatment, L-NAME and L-arginine groups (Fig. 3B). However, denudation of mucosa and atrophy of smooth muscle cells were seen in both the no-treatment and L-NAME groups after 8-wk obstruction (Fig. 3C). In contrast, the mucosa was intact and there was preservation of the hypertrophied muscle cells in the L-arginine-treated group after 8 wk (Fig. 3D).

Figure 4, A–E shows blood vessels immunostained with anti-CD31 antibodies in a section of normal rabbit bladder wall and the obstructed bladders. Figure 6A shows the quantitative vascular density measurements. The mean number of vessels per unit area was significantly increased equally among all subgroups after 2 wk of obstruction. However, by 8 wk after obstruction, vascular density decreased to control levels in the no-treatment group and was even lower in the L-NAME groups; yet the vessel density remained higher than the control level or the no-treatment group in the L-arginine group.

In the neurofilament studies, tissue from control animals demonstrated staining of nerve fibers of all sizes throughout the submucosa and muscularis (Fig. 5A). Qualitatively there was a decrease in small nerve staining in all the obstructed groups (Fig. 5, B–D). Quantitatively, the nerve density decreased after obstruction of 2 and 8 wk (Fig. 6B). However, at 8 wk after obstruction, the L-arginine-treated group had a nerve density that was significantly higher than the others.

![Image](https://example.com/image.png)

**Fig. 3.** Representative example of cross section of rabbit bladder wall from control, no treatment (A), 2-wk obstruction, no treatment (B), 8-wk obstruction, no treatment (C), and 8-wk obstruction, L-Arginine treated (D). Top, mucosa; bottom, muscle tissue sections from the different groups. Bladder wall looks similar among all control subgroups (A). Morphological changes after 2-wk obstruction all showed hyperplasia and hypertrophy of the smooth muscle among the no-treatment, L-NAME-, and L-arginine-treated groups. Tissues from L-NAME and L-arginine groups were similar to no-treatment groups and hence not shown (B). However, denudation of mucosa and atrophy of smooth muscle cell were characteristic in both no-treatment and L-NAME groups of 8-wk obstruction. Tissues from L-NAME group were similar to no treatment and hence not shown (C). On the contrary, intact of mucosa and preservation of muscle cell hypertrophy were well demonstrated in L-arginine-treated 8-wk obstruction (D). Reduced from magnification, x200. All were reduced equally.
Fig. 4. Vascular endothelium is immunostained with anti-CD31 antibodies. Reduced from magnification, ×40. All were reduced equally. A: blood vessels immunostained with anti-CD31 antibodies in a section of normal rabbit bladder wall (black arrows). Capillaries, as well as larger veins and arteries were stained. The normal serosa, characteristically, was one-cell thick. No blood vessels were present in the serosa. Bladder from 2- and 8-wk obstruction were characterized by serosal growth. Transmural angiogenesis occurred among all subgroups at 2-wk obstruction (B). Interestingly, at 8-wk obstruction, vascularization generally decreased in serosal and muscular layer in the no treatment (C) and L-NAME groups (D). Transmural angiogenesis was still found in the l-arginine group (E).

Fig. 5. Neurofilament staining of the bladder tissue sections. A: control bladder tissue section from no-treatment group showing a number of small and large nerve tracts (black arrows). B: in no-treatment group at 2-wk obstruction, there were decreases in small nerve tract staining (tissues from l-NAME and l-arginine groups were similar to no treatment and hence not shown). C: in no-treatment group at 8-wk obstruction, there were no small nerve tract staining, and there was a small decrement in the number of large nerve tracts (tissues from l-NAME group were similar to no treatment and is not shown). D: in l-arginine group at 8-wk obstruction, some small nerve fibers were well demonstrated. Magnification, ×200. All were reduced equally.
The CS activity in both mucosa (Fig. 7A) and muscle (Fig. 7B) decreased significantly among all groups at 2 and 8 wk after obstruction compared with control; on the other hand, compared with no-treatment group, the L-arginine group showed significantly higher activity after 8 wk of obstruction, thus correlating with the pattern of contractile responses.

In parallel to immunohistological finding, activity of ChAT also revealed a great drop in obstruction groups compared with control. However, again those premedicated with L-arginine had greater ChAT activity (Fig. 8).

In the studies on protein carbonylation and nitrotyrosine, the 2-wk obstructed groups responded virtually identically to the 8-wk obstructed groups and are not shown in the figures. Anti-DNP antibody detected a protein band at ~110 kDa in the mucosa and muscle of all groups, indicative of carbonylation (Fig. 9A and B). After 8 wk of obstruction, in both the no-treatment and L-NAME groups, there was significantly increased carbonylation in mucosa. In muscle, all three groups showed significant increases in carbonylation after 8 wk of obstruction. In contrast to the no-treatment group, the group premedicated with L-arginine showed significantly lower carbonylation compared with both no treatment and L-NAME.

There were 62- and 42-kDa bands detected with nitrotyrosine antibody in the bladder mucosa. The 62-kDa bands were virtually identical in all groups and are not shown in the figures (Fig. 10A), whereas, only the 62-kDa band was present in the smooth muscle (Fig. 10B). In the mucosa after 8 wk of obstruction, all treatment groups showed significantly increased nitrotyrosine (42-kDa band). However, the L-NAME showed a significantly higher level of nitrotyrosine than the no-treatment group.

**DISCUSSION**

Our earlier publications studied the acute effects of L-NAME and arginine using 1- and 2-wk obstructions (8, 38). These studies demonstrated clearly that both treatments had significant effects on bladder function via both changes in blood flow and changes in the generation of RNS free radicals. Since the bladder is a very dynamic organ, we have seen in many cases that the chronic effects of specific treatments can result in very different results from those of acute studies. We performed the current chronic studies using both L-NAME and arginine in the same experiment so that their effects could be directly compared. It was not surprising that some of the effects observed in the acute studies were maintained in the chronic studies, whereas other effects were very different.

In the current experiment, we used dietary L-arginine supplementation and administration of L-NAME to study the effects of NO on the response of the rabbit to chronic PBOO. The drug doses used were from our previous studies using these drugs. From our previous studies, we found that in long-term obstruction like 8 wk, sham-operated animals do not show significant differences from control animals and hence we had only controls for this study. We found a time course effect, with L-arginine and L-NAME. We found very little effects of L-arginine and L-NAME after 2 wk of obstruction.
However, after 8 wk of obstruction, the level of bladder decompensation was significantly greater for the L-NAME group compared with the no-treatment group, and the level of decompensation for the L-arginine group was significantly less compared with the no-treatment group. Thus, in general, with the longer time periods L-arginine was protective, whereas L-NAME was detrimental to bladder function.

Regarding the effect of L-arginine treatment, our study did not show a great reduction of the level of dysfunction at 2-wk obstruction, which was different from our previous study (38). We believe that this relates to the severity of the obstruction. The augmentation of bladder weight and magnitude of dysfunction in the previous study revealed a more severe obstruction that was in contrast to the milder, but prolonged obstruction created in this study for the purpose of observing the chronic effects. Regardless of the severity of obstruction, the L-arginine group ultimately showed significant reduction in the level of bladder dysfunction, similar to our previous study (38).

We found that L-NAME showed a minimal effect on bladder contractile properties and histological appearance after 2 wk of obstruction and was detrimental to bladder function after 8 wk of obstruction. In a previous study, we demonstrated that L-NAME inhibition of NOS reduced bladder deterioration of bladder function after 3 and 7 days of obstruction and thus suggested a beneficial effect on I/R injury (8). This seemingly paradoxical finding can be explained by the different time periods of PBOO (7 days vs. 8 wk), as well as differences between the in vivo I/R model and the chronic PBOO model (30–32).

In ours and other studies, there is a rapid increase in NOS activity immediately following PBOO, which results in the generation of nitrotyrosine, thus resulting in significant short-term RNS damage. Pretreatment with L-NAME results in the blockade of RNS generation and is thus protective of reperfusion damage in the short-term studies (7 days and less) (8). Alternately, the inhibition of NO production by L-NAME during chronic PBOO would not be a major factor in preventing the obstructive injury. One of the major roles of NOS in the bladder is to control blood flow. Thus, L-arginine can result in an increase in blood flow, while L-NAME can result in decreased blood flow (8, 38).

We also demonstrated in both bladder weight and contractile properties that L-arginine treatment prevented further deterioration at 8 wk compared with L-NAME. Our study supports this idea via the blood-vessel studies, where the level of angiogenesis was similar at 2 wk following obstruction for all groups, but was significantly increased by L-arginine after 8 wk of obstruction and significantly decreased by L-NAME after 8 wk. Studies have shown that NO promotes new vessel growth through NO-mediated upregulation of angiogenic factors, such as vascular endothelial growth factor and basic fibroblast-derived growth factor (7). NO also affects the proliferation of vascular cells, such as endothelial and smooth muscle cells through the cGMP signaling pathway (33, 24). Decreased blood flow to bladder smooth muscle is an etiological factor in bladder contractile dysfunction (bladder de-

![Fig. 7. Effect of L-arginine and L-NAME on citrate synthase (CS) activity in mucosa (A) and muscle (B). The CS activity in both mucosa (Fig. 6A) and muscle (Fig. 6B) significantly decreased among all groups at 2-wk obstruction and 8-wk obstruction compared with control; on the other hand, compared with no-treatment group, L-arginine group showed significantly increased activity at 8-wk obstruction, correlating in time with the contractile responses. Each bar is the mean ± SE of 4 individual rabbits. *Significantly different from control (P < 0.05); †significantly different from no-treatment group (P < 0.05).

![Fig. 8. Effect of L-arginine and L-NAME on choline acetyltransferase activity in muscle. Each bar is the mean ± SE of 4 individual rabbits. *Significantly different from control (P < 0.05); †significantly different from no-treatment group (P < 0.05).]
Therefore, angiogenesis due to supplementation of l-arginine could well compensate for the ischemia mediated by obstruction and prevent or delay progressive deterioration of the bladder function secondary to chronic PBOO. On the other hand, inhibition of angiogenesis by L-NAME administration would make ischemic damage and bladder contractile dysfunction worse, which is also reflected in the marked augmentation of bladder weight at 8-wk obstruction. Since bladder weight has been demonstrated to be an excellent indicator of the...
severity of obstructive dysfunctions (14, 25), we believe that this confirms the protective effect of L-arginine.

Denervation in the obstructed bladders was estimated by means of neurofilament staining and a ChAT functional assay. It is the small nerve tracts that innervate the bladder smooth muscle tissues. During bladder hypertrophy due to obstruction, the smaller nerve tracts degenerate, and we have seen a decrease in small nerve staining. Denervation may also account for the decreased contractile function in chronic obstruction. L-Arginine supplementation appeared to be neuroprotective in chronic PBOO; L-NAME administration did not show effect of neuroprotection in bladders after 2 and 8 wk of obstruction, a finding that was again different from previous study in 7 days of PBOO (8). Both carbonylation and nitrotyrosine at 8 wk of obstruction were lower in L-arginine group compared with the non-treatment group. Carbonylation and nitrotyrosine are generated from the reaction of the ROS and RNS with protein, respectively, and are associated with neuronal ischemic injury and numerous neurodegenerative diseases (35, 16). Therefore, we believe that L-arginine limits formation of oxidatively modified protein in chronic PBOO, promoting neuronal survival and preventing denervation. In support of this are findings that L-arginine could ameliorate oxidative stress and enhance functional recovery after I/R in heart (9, 37).

One of the markers for bladder dysfunction is mitochondrial dysfunction (22). Consistent with our results of contractile properties and bladder weight in 2 and 8 wk PBOO, CS activity marked a difference only at the 8-wk treated L-arginine group compared with non-treatment group. Because PBOO of the rabbit induces a shift from aerobic to anaerobic metabolism, dysfunction of the bladder correlates with the decreased cellular concentration of high-energy phosphates generated through oxidative metabolism (14, 15). The cellular mechanism responsible for decreased oxidative metabolism involves decreased mitochondrial enzyme activity, such as decreases in the activity of CS (12). Higher CS activity at L-arginine-treated 8-wk PBOO further supports our theory via increased vasculature that oxidative metabolism was improved, preventing the less-efficient anaerobic metabolism and improving bladder function.

There is no doubt that arginine results in a marked increase in NO synthesis and thus would also result in an increased generation of RNS free radicals. However, simultaneously the increased NO synthesis and release increases blood flow to the bladder and decreases the level of hypoxia of the obstructed bladder. The increased blood flow would significantly improve mitochondrial function, thus reducing the detrimental effects of both hypoxia and reperfusion, and reduce the generation of mitochondrial-generated free radicals (ROS).

Alternately, L-NAME would inhibit NO synthesis and thus reduce the direct generation of RNS. However, L-NAME would also prevent NO-stimulated increases in blood flow and thus enhance the level of hypoxia and reperfusion damage, resulting in increased ROS generation from the compromised mitochondria. The results of our studies clearly show that the effects of NO on blood flow are significantly more important than the effects on RNS generation, thus showing arginine much more effective in improving obstructed bladder function than L-NAME.

Other studies have explored the relationship between NO with PBOO by means of investigating the roles of NOS. Three distinct isoforms of NOS, inducible NOS, endothelial NOS (eNOS), and neuronal NOS have been identified. Both Lemack et al. (17, 18) and Felsen et al. (10) suggested that pharmacological or genetic decreases in inducible NOS resulted in amelioration of functional and fibrotic changes in the bladder after PBOO. On the other hand, there are many studies that show that eNOS plays a totally opposite role in I/R injury because the upregulation of eNOS protects against I/R injury under pathophysiological conditions in other organs (2, 4, 5, 13, 32a). Decrements in eNOS activity and/or expression are found in many disease states and are thought to be an important mediator in the pathogenesis of these diseases, including hypertension, hypercholesterolemia, and atherosclerosis (2). Although we did not measure the activity of each isoform of NOS in our study, this would be an excellent topic for future studies.

The present study has some limitations. One is the fact that we only studied two time points, limiting the assessment of ongoing changes. Also, because we premedicated the animals, we do not know whether this is necessary, and, moreover, we have no data on how long L-arginine administration should be continued. In addition, actual measurements of blood flow would be more direct in determining blood flow than histological study of the vessels and our measurement of oxidative stress.

Nonetheless, in contrast to the beneficial effect of L-NAME on high-grade short-term obstruction, supplement of L-arginine, a NOS substrate, ameliorated contractile dysfunction after chronic PBOO. Associated with this improvement were increases in vascular and nerve density and a reduction in carbonylation and nitrotyrosine generation, signs of less oxidative stress. These suggest mechanisms of action of L-arginine in this animal preparation.

Perspectives and Significance

In this study, we investigated the influence of NOS activity on the response of rabbits to chronic PBOO. Our results demonstrated that increasing blood flow by stimulating NO by pretreating the rabbits with L-arginine significantly protected the bladder from PBOO dysfunctions, whereas inhibiting blood flow by L-NAME enhanced the dysfunctions mediated by PBOO. Future studies will be directed toward identifying which specific proteins are nitrated and carbonylated during outlet obstruction using proteomic approaches.

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