Sex differences and role of nitric oxide in blood flow of canine urinary bladder

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Pontari, Michel A., and Michael R. Ruggieri. Sex differences and role of nitric oxide in blood flow of canine urinary bladder. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R407–R413, 1999.—Continuous measurements were made of bladder blood flow by laser Doppler flowmetry in anesthetized dogs during bladder filling and emptying. In both mucosa and muscle, perfusion was inversely proportional to intravesical pressure. There was significantly greater perfusion in the bladder mucosa of males than females at baseline and up to 10 cm water filling pressure but not in the muscle. Intra-arterial infusion of the nitric oxide synthase inhibitor N^G-nitro-L-arginine produced a significant decrease in resting bladder perfusion in the mucosa only, with no differences seen in the response to intravesical pressure. Intra-arterial infusion of L-arginine produced a significant increase in the level of perfusion in the mucosa seen immediately after the bladder was drained. No changes were observed in muscle perfusion after L-arginine. These results suggest that the perfusion of the bladder mucosa differs by gender and is regulated differently than the bladder muscle, possibly related to the different function of the two layers.

perfusion; cystometry; laser Doppler flowmetry; N^G-nitro-L-arginine; nitric oxide synthase

BLADDER PERFUSION has been proposed as one possible factor in the pathogenesis of urinary tract infections (7), the bladder changes associated with outlet obstruction (3), and, more recently, interstitial cystitis (IC) (15). Whereas older techniques using microspheres could produce only static measurements, the use of laser Doppler flowmetry has allowed for the real-time measurement of bladder blood flow. Previous studies continuously monitoring bladder perfusion and changes with the micturition cycle have examined the detrusor muscle (3, 4, 9). Recent evidence suggests that the muscle and mucosal vascular beds may be anatomically distinct (11) and that the muscle and mucosa are metabolically different (13). The purpose of this study is to examine the effect of bladder filling and emptying on bladder mucosal and muscle perfusion measured simultaneously in the same bladder. Given that 90% of patients with IC are female, we included both male and female dogs to see if there are sex differences in bladder perfusion that may contribute to the pathogenesis of the condition and help account for this disparity in prevalence.

The factors that modulate bladder blood flow are poorly understood. As assessed by measuring venous outflow in cats, bladder vasodilation is reduced but not abolished after administration of indomethacin (1). In the same study, the flow response to filling was unaffected by cholinergic or adrenergic blockade and not associated with a significant change in the concentration of substance P or vasoactive intestinal polypeptide. In the dog, sympathetic nerve stimulation produces an increase in blood flow in the bladder neck, but not bladder body or trigone (3, 10). Pelvic nerve stimulation results in bladder contraction, with bladder blood flow reported as either decreased (3) or unchanged (10). Nitric oxide (NO) has been reported to be involved in the control of perfusion in a wide range of vascular beds, including coronary, cerebral, renal, duodenal, and systemic resistance vessels that control blood pressure (8, 12, 28). The role of NO in bladder perfusion is unknown. A second purpose of this study is to determine if NO is involved in the regulation of bladder perfusion and to assess any differences in regulation between the bladder muscle and mucosa.

MATERIALS AND METHODS

A total of 18 dogs, 9 female and 9 male, weighing between 20 and 30 kg were used in this study. Measurement of perfusion in bladder muscle and mucosa during bladder filling and emptying. For the measurements of perfusion of fluid and by bladder filled, 10 dogs, 5 female and 5 male, were used. Anesthesia was induced with 30 mg/kg pentobarbital sodium intravenously and maintained with additional doses as required. The animals were intubated via an endotracheal catheter to maintain an open airway. An intravenous solution of 0.9% NaCl was infused at 125 ml/h. Systemic blood pressure was monitored via a 20-Fr cannula placed in the right carotid artery. After a midline laparotomy, a cystotomy was made for placement of both a suprapubic tube and a mucosal blood flow probe. Intravesical pressure was measured via an 18-Fr three-way Foley catheter that was passed through the dome of the bladder and sutured in place. One port was connected to a pressure transducer, and the other was used for instillation of normal saline into the bladder.

Continuous measurement of bladder blood flow was made by laser Doppler flow probes (Perimed, Järfälla, Sweden). The incident laser light in the mucosal blood flow probe (PF 418) emanates from the center of a 1-cm-diameter disc at right angles to the long axis of the laser fiber. This disc has eight holes around its periphery that were used to sew the probe directly onto the mucosa. The muscle blood flow probe (PF 404) was either used directly or fitted with a bare fiber extension that was placed through the serosa into the muscularis. Establishing that different vascular beds were being monitored by the two different probes was done in two ways. The mucosa was removed, and the mucosal flow probe was placed against the underlying muscle that produced lower perfusion
readings. In a separate experiment, the removed mucosa was
interposed between the mucosal probe and the intact bladder
mucosa, which eliminated perfusion readings from the under-
lying mucosa. This confirmed that the PF418 probe was
actually measuring mucosal blood flow and not flow in the
underlying muscle. The laser Doppler signal was sampled
and recorded at 32 Hz. Continuous readings of systemic blood
pressure, muscle perfusion, mucosal perfusion, and bladder
pressure were recorded by a Texas Instruments 4000M laptop
computer. The systemic and bladder pressure were channelled
first through a physiological recorder (Grass model 7D poly-
graph; Grass Instruments, Quincy, MA). Perfusion measure-
ments were continuously recorded by the computer using
custom software (Perimed).

The bladder was filled at 30 ml/min, and measurements of
perfusion were made at either the dome or base first, which
were alternated in each dog. Three to four filling runs were
made at each location per dog. Bladder blood flow and
intravesical pressure were recorded as baseline at the point
at which the values were stable before the start of the experi-
mental condition. The value for drain represents the
peak value after emptying the bladder that occurred within
15 s after bladder emptying.

NO studies. The effect of the NOS inhibitor N\textsuperscript{G}-nitro-L-
arginine (L-NNA) and L-arginine (L-Arg) on bladder perfu-
sion was studied in eight additional dogs, four female and four
male. Bladder blood flow probes and monitoring of systemic
and intravesical pressure were performed as above except
that muscle perfusion was measured by the blood flow probe
(PF 404) without the extension fiber. For these studies, flow
probes were placed along the back wall of the bladder, near
the junction of dome and bladder base. A 5-Fr cardiac access
sheath was placed into the left femoral artery of each animal.
Drugs were infused through this access. The internal infusion
sheath through which drugs were injected extended to the
bifurcation of the aorta, as confirmed surgically. L-NNA was
dissolved in acidified saline (pH = 2), and L-Arg was dissolved
in saline (pH = 7). The bladder perfusion was allowed to
equilibrate to a stable baseline. A filling cycle was then
performed as above, with saline instilled at 30 ml/min. In five
animals, acidified saline, the vehicle for L-NNA, was given
intra-arterially after a stable baseline was obtained and
before the filling curve. After an intra-arterial administration
of 20 mg/kg L-NNA through the femoral artery access sheath,
the effect on baseline perfusion was observed for 5 min. A
filling cystogram was then repeated using saline at 30
ml/min. This sequence was then repeated using 200 mg/kg
L-Arg. The values for baseline blood flow after drug adminis-
tration represent the maximal change during the 5-min
observation period. L-NNA and L-Arg were purchased from
Sigma (St. Louis, MO).

Statistics. Differences in bladder perfusion with changes in
intravesical pressure were assessed using repeated-measures
ANOVA with post hoc Newman-Keuls pairwise comparisons
or Student’s t-test where appropriate.

RESULTS

Measurement of perfusion in bladder muscle and
mucosa during bladder filling and emptying. A represen-
tative tracing of the mucosal and muscle blood flow as
measured by laser Doppler flowmetry, along with blad-
der pressure, is shown in Fig. 1. The basic response of
bladder perfusion on filling is a decrease with increas-
ing intravesical pressure. This was observed in both
muscle and mucosa (Fig. 2). However, the rate of the
decrease in perfusion was less in the mucosa, especially
in female animals. After emptying the bladder with the
resultant reduction in intravesical pressure, there is a
compensatory overshoot of the perfusion in the muscle
before return to near baseline levels. The mucosa
perfusion increased back to baseline levels but did not
appear to overshoot baseline as did the muscle. Perfu-
sion units obtained from the laser Doppler flow probes
are arbitrary and are not directly comparable between
different probes. However, the relative change in perfu-
sion as measured by different probes can be directly
compared (Fig. 2, bottom).

There was a statistically greater baseline perfusion in
the mucosa of male bladders compared with that of
female animals (Fig. 2, top left). This difference was
significant up to 15 cmH\textsubscript{2}O pressure. There was no
difference in levels of perfusion for the muscle between
sexes or in bladder perfusion between the dome and
base of the bladder for either muscle or mucosa in
either sex (results not shown).

NO studies. The intra-arterial injection of acidified
saline (vehicle for L-NNA) had no effect on baseline
blood flow in muscle or mucosa (Fig. 3) or on the
response to intravesical pressure (Fig. 4). L-NNA pro-
duced a statistically significant decrease in basal muco-
sal blood flow (P < 0.05) but had no effect on basal
muscle blood flow. L-Arg had no significant effect on
basal blood flow in either layer. With filling, the curves
after L-NNA or L-Arg were not significantly different
from the standard filling curves performed in the
absence of drug for muscle or mucosa (Fig. 4). The
perfusion in the mucosa after bladder drainage was
significantly greater after L-Arg than after L-NNA or
saline infusion (P < 0.05). This difference was not
observed in the muscle.

DISCUSSION

The continuous measurement of bladder mucosal
blood flow has not been previously reported. In general,
the bladder mucosa demonstrates a response similar to
the bladder muscle, i.e., perfusion is inversely propor-

![Fig. 1. Representative tracing of response of bladder perfusion to
to changes in intravesical pressure. Tracing is taken from a female
animal. Bladder perfusion is in arbitrary units recorded continu-
ously from laser Doppler flow probe. Filling rate was 30 ml/min. Record-
ing was taken at bladder dome. Scale bar: muscle perfusion, 10 perfu-
sion units; mucosa perfusion, 50 perfusion units; bladder pressure, 10 cm
water pressure.](http://ajpregu.physiology.org/
ional to intravesical pressure. On bladder drainage with the acute reduction of intravesical pressure, mucosal blood flow returns to baseline levels, whereas a transient overshoot of baseline levels is observed in the muscle (Fig. 2).

A direct comparison of the amount of perfusion in the mucosa and muscle is not possible in our study because different fiber optic probes were used for the two different sites. The arbitrary perfusion units from two different laser Doppler flow probes are not directly comparable, due to differences in geometry of the probes. Therefore, we can make no determination of which bladder compartment has greater perfusion. Relative changes can be compared, however, and are presented in Fig. 2, bottom. Two previous studies using radiolabeled compounds found greater perfusion in the mucosa, ranging from a 2:1 ratio with the bladder distended to 13:1 with the bladder empty (7, 24). One study reported a greater blood flow in the muscle than in the mucosa in the resting, empty state, by a 2:1 margin (3).

Our results with response of the bladder muscle blood flow to filling are consistent with earlier studies that used indirect methods of assessing bladder perfusion. Studies using radiolabeled washout techniques have demonstrated a similar inverse relationship between bladder muscle blood flow and filling (4, 7). In other studies using the laser Doppler technique, bladder muscle blood flow decreased to 60% (3) and 45% (9) of baseline levels at maximal capacity, corresponding to 20–25 cm H₂O pressure in the unobstructed bladder. This range is similar to our findings that muscle blood flow in both males and females decreased to ~50% of baseline perfusion at the same intravesical pressure. We found no difference in the perfusion at the bladder base compared with the dome in either the mucosa or muscle. Other studies have reported either no difference in blood flow between the bladder base and dome (24) or greater perfusion at the base than dome in the bladder muscle (3).

In the muscle perfusion, with the rapid decline in intravesical pressure with bladder drainage, there was a transient overshoot of resting basal levels of perfusion before return to baseline. This was also reported by Azadzoi et al. (3) and is visible in the data presented by Greenland and Brading (9). This hyperemic response after a reduction in blood flow has been observed in the heart (19). This same response was not evident in the response of the bladder mucosa after release of intravesical pressure (Fig. 2). The hyperemia may be one way to correct for the transient ischemia produced by bladder filling.

Our results showing a decrease in bladder mucosal perfusion with increasing pressure are consistent with noninvasive studies (7, 24). The relative change of mucosa to muscle with increasing pressure has previously been reported as being similar. Finkbeiner and Lapides (7) found that both decreased to a similar amount (27 vs. 31%) with 2 h of distension. Our curves (Fig. 2) suggest that the mucosa perfusion is somewhat more resistant to increasing intravesical pressure than that in the muscle. This may reflect a mechanical phenomenon, in that with filling there is more redundant mucosa that must be filled out before the intravesical tension is applied evenly along the mucosal surface. Alternatively, this may reflect differences in the blood flow regulatory mechanisms between the two layers.

The difference in filling among mucosa and muscle is most visible in the mucosa of female animals. There is a significantly greater level of perfusion in the male...
compared with the female mucosa at baseline and up to 15 cmH₂O water pressure of filling. Beyond that point, the absolute levels of bladder perfusion are not significantly different. The end result is that in addition to differences seen at baseline, the blood flow in the female mucosa does not decrease to the same degree in the face of increasing intravesical pressure. A lower resting blood flow in the female bladder has been reported in the bladder of the female rabbit; however, the mucosa and muscle blood flow were not distinguished (22). In the same study, gender differences were also found in many other organs, including those in which the blood flow was greater in the female (sternum, intercostal muscles, spleen) as well as deceased in the female (skin of toe, ear, nasal turbinate). Gender differences in different parts of the same organ have been reported in the kidney (25). Decreased bladder mucosal perfusion has been suggested as one contributing factor to the pathogenesis of IC, a condition in which 90% of affected individuals are female. Differences in bladder blood flow between genders may reflect differences in factors regulating perfusion. Gender differences in both α- and β-adrenergic receptors have been noted in the bladder and urethra of rabbits (21). Estrogen has been shown to diminish vascular resistance to blood flow in a number of vascular beds, and the vasodilator effects of estrogen can be reversed by inhibitors of NO synthesis, indicating that NO mediates this vasodilation (6). Prostaglandins are released into the pelvic venous blood in dogs with vesical distension and micturition (17). Sex differences in the role of prostaglandins on local perfusion have been reported in the kidney, where indomethacin reduces medullary blood flow in female but not male rats (25).

Our results also show a difference in response of the basal blood flow between the mucosa and muscle of the dog bladder after administration of the NO synthase (NOS) inhibitor L-NNA. L-NNA has been shown to be a potent inhibitor of endothelial NOS (23). The effect of NO on bladder perfusion has not previously been reported. NO is known to play a role in the perfusion of a wide range of vascular beds, including coronary, cerebral, renal, duodenal, and systemic resistance ves-

**Fig. 3.** Effect of nitric oxide synthase inhibitor and L-arginine (L-Arg) on basal bladder perfusion and mean arterial blood pressure; n = 8 for NO-nitro-L-arginine (L-NNA; 20 mM) and L-Arg (200 mM) and n = 5 for L-NNA vehicle, acidified saline, pH = 2. All drugs are given intra-arterially. Perfusion is in arbitrary perfusion units. Bladder recordings are at an area of junction of dome and base along back wall of bladder. *P < 0.05.

**Fig. 4.** Response of bladder mucosa and muscle to increasing intra-vesical pressure after intra-arterial administration of nitric oxide synthase inhibitor and L-Arg; n = 8 for L-NNA (20 mM) and L-Arg (200 mM). All drugs are given intra-arterially. Saline curves represent combined first filling curves in 8 dogs, 5 after intra-arterial acidified saline (pH = 2, L-NNA vehicle) and 3 with no arterial infusion, because acidified saline was found to have no effect on response to filling. Perfusion is in arbitrary perfusion units. Bladder recordings are at an area of junction of dome and base along back wall of bladder. Filling rate is 30 ml/min. Drain denotes 0 pressure immediately after bladder is drained after filling. *P < 0.05.
sels that control blood pressure (8, 12, 28). The response to NOS inhibitors is not uniform and varies by species, organ, and even site in the organ (8). L-NNA produced a decrease in resting bladder blood flow in the bladder mucosa but not the muscle. NOS inhibitors in vitro produce endothelium-dependent and enantiomer-specific contraction of vascular rings, confirming that there is a continuous use of L-Arg for the basal release of NO (27). Intravenous infusion of N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME) in some species produces an increase in blood pressure (8, 28), indicating that there is an inhibition of basal release of NO by the endothelium of resistance vessels that may contribute to blood pressure control in these species. Inhibition of basal NO synthesis in the bladder mucosa with L-NNA results in a decrease in bladder blood flow. Therefore, we conclude that NO contributes to the basal perfusion in the bladder mucosa.

The difference in effect of NOS inhibitors in different parts of one organ has been seen previously in the heart and kidney. Intravenous L-NAME given to dogs produces a selective reduction in left ventricular blood flow in the subepicardial layers only and a reduction in blood flow in all layers of the right ventricle (33). In the kidney, the addition of L-NNA has different effects on different vessels. L-NNA has no effect on the main arcuate artery but vasoconstricts the interlobular arteries when blood pressure increases (14). The magnitude of the decrease in bladder mucosal blood flow (25%) is consistent with the reduction in blood flow seen in other organs after systemic NOS inhibitors in vivo (16, 33).

There are several possible explanations for the difference seen between mucosa and muscle. One may be differences in the L-arg/NO system in the two different vascular beds, possibly due to anatomic differences in the vessels themselves. The vasculature of the bladder mucosa has been described as a dense collection of capillary vessels associated with folds in the basal layer next to the epithelial cells themselves, whereas the muscle layer contains larger vessels (31). There is evidence that the importance of the L-Arg/NO pathway in vasodilation varies with vessel size. In the heart, topical N<sup>G</sup>-methyl-L-arginine (l-NMA) has been reported to constrict small (<120 µm) arterioles but not larger (>120 µm) arterioles (18). The results may reflect the relative importance of the NO pathway in vasodilation in the two layers. It is possible that other local factors controlling blood flow such as neural mechanisms may be different in the mucosa and muscle. The role of NO in a particular tissue may also change under different circumstances. Changes in perfusion pressure can produce different responses to L-NNA (14). Thus local environment may influence the significance of the NO contribution to regulating perfusion.

The decrease in perfusion seen in the bladder mucosa after injection of L-NNA may involve more than just the inhibition of local production of NO. L-NMA infusion in anesthetized dogs results in a decrease in renal blood flow and is associated with an increase in plasma endothelin (26). NOS inhibitors when given systemically also cross the blood-brain barrier and can enhance sympathetic activity by a central action (31). Thus both endothelin and interaction with the sympathetic nervous system may be other factors in our observed decrease in resting mucosal bladder blood flow after intra-arterial L-NNA.

We saw a small increase in mucosal perfusion after intra-arterial L-Arg, but this was not statistically significant. No change was seen after L-Arg in the muscle. The effect on regional perfusion of L-Arg given after an NOS inhibitor differs in different vascular beds (8). Our results are similar to other studies that show a lack of effect of L-Arg either in vitro on vessel vasodilation or in vivo on basal renal perfusion (20, 27). This would indicate that L-Arg availability is not rate limiting for basal release of NO in the bladder vasculature, either because exogenous L-Arg cannot reach the site of action or because the NOS enzyme is already saturated with endogenous L-Arg. It is also possible but unlikely that the dose of arginine given, 200 mg/kg or 10 times that of the L-NNA, was insufficient to overcome L-NNA inhibition, because a fivefold excess of L-Arg completely reverses the effects of L-NNA in vitro (23). Because blood pressure decreased after the L-Arg but blood flow did not, the vascular resistance (MAP/blood flow) decreased. It is possible therefore that there is some vasodilation from the L-Arg producing a maintenance of perfusion in the face of a decreased blood pressure. Also, in our experiments, L-Arg was given after a cycle of bladder filling and emptying after the L-NNA. There may be vasoactive substances released with bladder filling (17) that modify the effect of L-Arg on resting bladder blood flow.

With bladder filling, we observed no significant change in the response of perfusion to intravesical pressure after L-NNA or L-Arg (Fig. 4). There was a significant difference seen in the level of perfusion after bladder drainage in the mucosa after L-Arg compared with L-NNA. No difference was observed in the muscle. These results are consistent with recruitment of L-Arg and release of NO from the mucosa during bladder drainage and the resultant transient hyperemia. In situations in which L-Arg is mobilized during stimulation by agonists such as acetylcholine, it takes more NOS inhibitor to block acetylcholine-induced vasodilation than to block basal NO synthesis and affect resting blood pressure (27). With stimulation of NOS by acetylcholine, L-Arg potentiates the response to acetylcholine in situations in which it has no effect on resting tone (32). This suggests that L-Arg availability is limiting when NO synthesis is enhanced by acetylcholine but not under basal conditions. This is supported by findings that L-Arg uptake is stimulated by agonist-induced NO synthesis (5). Our results are similar to those seen with agonist-induced uptake of L-Arg and release of NO and are consistent with L-Arg uptake and release of NO by the bladder mucosa as the bladder is drained. The effect is possibly not inhibited by L-NNA because enough L-Arg is being taken up for NO production and release to overcome L-NNA for binding sites on NOS. The vasodilator effect of the NO is augmented by...
exogenous L-Arg because it is being actively recruited for NO production.

Two reasons that NO might be expected to be liberated during the hyperemic response seen with bladder drainage after filling are an increase in flow and transient hypoxia. The bladder perfusion increases rapidly and markedly when the intravesical pressure is reduced by bladder drainage. NO has been shown to be released from blood vessels by an increase in flow (29). Also, during bladder filling, the bladder has been shown to become temporarily hypoxic (3). In the heart, hypoxia causes an increase in myocardial blood flow that is mediated by NO (2). NO has been shown to mediate the hyperemic response seen in the heart that follows as little as 200 ms of coronary occlusion (19). Therefore, NO may also be released in the bladder as a response to tissue hypoxia. Other factors may also be responsible for the hyperemia. During reactive hyperemia in the heart, adenosine is released and the amount is significantly increased in the presence of L-NAME (19). It is possible that blockade of NOS induces other compensatory vasodilator pathways to maintain the perfusion and hyperemia in the bladder.

Whether NO is released during bladder filling is not clear. We did not observe a significant change in the perfusion response with either L-NNa or L-Arg. If NO is released with filling, we would not expect to see a decrease in perfusion after giving L-NNa if there is active uptake of L-Arg in quantities sufficient to overcome the competitive inhibition of L-NNa. Also, other vasoactive substances such as prostaglandins are released with vesical distension (17). Even if L-NNa inhibited NO release with filling, the release of other vasodilator substances may maintain perfusion in the face of increasing intravesical pressure. If L-Arg is recruited for NO release with filling, then addition of L-Arg may be expected to augment this response. This is supported by the response of the mucosa to filling after intra-arterial L-Arg, especially at 10 cmH2O pressure (Fig. 4). However, the predominant factor in controlling bladder perfusion appears to be intravesical pressure. Therefore, it is possible that even if NO is released, the vasodilator effects are outweighed by the effects of increasing intravesical pressure.

Our results indicate differences in blood flow between males and females and a role for NO in both basal bladder perfusion and reperfusion after the relative ischemia produced by bladder filling. All of these were seen exclusively in the bladder mucosa and not the muscle. The bladder mucosa and muscle are metabolically different. The mucosa has a higher rate of glucose metabolism, greater activity of mitochondrial citrate synthase, and a greater ratio of anaerobic/aerobic metabolism, indicating that the mucosa is more metabolically and energetically active under basal conditions than the muscle (13). The functions of the two layers of the bladder are distinct. In addition to other functions, the mucosa is involved in antibacterial adherence and impermeability. Specific anatomic characteristics facilitate these functions in the mucosa (11), and both are compromised by anoxia and ischemia (30).

half-life of NO is inversely proportional to oxygen tension (16), this may be an important regulatory mechanism in maintaining bladder mucosal perfusion in the face of hypoxia and maintaining mucosal function.

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