J Pharmacol Sci **95**, 305 – 310 (2004)

*Journal of Pharmacological Sciences*


*Full Paper*

# Doxazosin Effects on Cholinergic and Adrenergic Responses in Rat Isolated Detrusor Smooth Muscle Preparations From Obstructed Bladder

Coşkun Usta[1,*], Erdal Kukul[2], and Mehmet Yalçınkaya[2]

[1]*Department of Pharmacology and* [2]*Department of Urology, Akdeniz University Faculty of Medicine, 07070 Arapsuyu, Antalya, Turkey*

*Received May 29, 2003; Accepted April 21, 2004*

**Abstract.** We investigated the effect of doxazosin on cholinergic and adrenergic agonists responses in detrusor smooth muscle preparations from sham-operated and 2-week partially obstructed rat bladders. Male Wistar albino rats, 200 – 250 g, were randomly allocated to 4 experimental groups consisting of 12 animals each: sham-operated bladder, sham-operated bladder treated with doxazosin, partially obstructed bladder, and partially obstructed bladder treated with doxazosin. Partial outlet obstruction of the rat was surgically induced. The response to carbachol ($10^{-7}$ – $10^{-4}$ M), isoproterenol ($10^{-6}$ – $10^{-3}$ M), and 80 mM KCl were recorded. Carbachol caused concentration-dependent contractile responses in the detrusor smooth muscles from sham-operated and partially obstructed bladder. Isoproterenol produced concentration-dependent relaxation responses in the detrusor strips from all groups. Dose-response curves for carbachol and isoproterenol showed a shift to the left in rat detrusor smooth muscles from partially obstructed bladder when compared with the results obtained in detrusor muscles from sham-operated bladder. These responses were reversed to normal by doxazosin treatment in rat detrusor smooth muscles from partially obstructed bladder. KCl produced contractile responses in rat detrusor smooth muscles from all groups. The contractile responses to KCl were not significantly changed in all groups. We have shown that carbachol and isoproterenol responses were shifted to the left in rat detrusor smooth muscles from partially obstructed bladder and these responses were reversed by doxazosin treatment.

***Keywords***: carbachol, isoproterenol, alpha blocker, detrusor smooth muscle

## Introduction

Bladder outlet obstruction is a common medical problem. About 80% of elderly males have various degrees of urinary bladder outlet obstruction secondary to benign prostatic hyperplasia, prostatic cancer, bladder stones, uretral strictures, and bladder cancer. The urinary bladder is responsible for two important physiological functions: urine storage and urine emptying. An effective and sustained detrusor muscle contraction is essential for bladder emptying.

Bladder outflow obstruction results in major alterations in bladder structure and function, including detrusor hypertrophy, elevated voiding pressures, and detrusor instability. The effects of bladder outflow obstruction have been studied in several animal models. The pathophysiological mechanisms underlying these conditions are not understood. The urinary bladder is innervated by both sympathetic and parasympathetic fibers via the hypogastric and pelvic nerves. The parasymphatic nervous system plays an important role in functional regulation of bladder smooth muscle. Parasympatheticnervation is responsible for detrusor contraction. Some studies have shown that there were changes in bladder smooth muscle responses to cholinergic agonists in obstructed pig bladders. Sympathetic innervations also play an important role in bladder smooth muscle and are responsible for detrusor relaxations (1). Recently, much attention has been

*Corresponding author. FAX: +90 242 2274482
E-mail: fccos@msn.com

focused on the role of the sympathetic nervous system and alfa-1-adrenoreceptors ($\alpha$1AR) in the smooth muscle contraction component of bladder outlet obstruction (2, 3). $\alpha$1AR antagonists have been demonstrated to relax prostate smooth muscle and they were capable of relieving outlet obstruction in clinical studies (4 – 6). Therefore, in this study, we investigated the effect of doxazosin on cholinergic and adrenergic agonist responses in rat detrusor smooth muscle preparations from sham-operated bladder and 2-week partial obstructed bladders.

## Materials and Methods

*Treatment of animals*

After obtaining University animal care committee approval, 48 male Wistar rats weighing 200 – 250 g were used in this study, randomly allocated to 4 experimental groups consisting of 12 animals each: sham-operated bladder, sham-operated bladder treatment with doxazosin, partially obstructed bladder and partially obstructed bladder treatment with doxazosin. They were individually housed in plastic cages in a quiet, temperature- and humidity-controlled room (22 ± 5°C and 63 ± 5%, respectively) in which a 12 h – 12 h light-dark cycle was maintained (lights on between 08 – 20 h). Animals weights were recorded every day.

*Surgical procedures*

For inducing partial obstruction of the urinary bladder, firstly 24 rats were anesthetized with an intraperitoneal injection of 40 mg/kg pentobarbital, and then the abdomen was opened via low midline incision and the prostate lobes were retracted bilaterally to expose the bladder and the bladder neck. After removal of the perivesical fat, bilateral dissection of the proximal urethra was performed and a 2-0 silk suture was passed between the urethra and rectum and placed around the urethra over a catheter (1.70 mm). Then the urethra was ligated and the catheter was removed. The catheter ensured that the ligature did not completely close the urethra, but reduced its diameter to nearly 2/3 of the original size. The incisions were closed with surgical suture and the operated rats were housed in separate cages for 2 weeks. Sham surgery was performed in 24 additional rats except that urethral ligatures were removed instead of being tied in place. Each rat was placed in a recovery room and observed for several hours. Intramuscular gentamicin (1 mg/kg) and tramadol hydrochloride (20 mg/kg) were given during postoperative days. Some of the sham-operated and some of the obstructed animals received 1 mg/kg per day doxazosin mesylate, administered by an intraperitoneal injection for 2 weeks.

*Pharmacological studies*

At the end of 2-week period, the urinary bladders above the level of the urethral orifices were excised and placed in a physiological saline solution (PSS) of the following composition: 123 mM NaCl, 5.9 mM KCl, 1.2 mM $MgCl_2$, 15.5 mM $NaHCO_3$, 1.2 mM $KH_2PO_4$, 1.25 mM $CaCl_2$, and 11.5 mM glucose, gassed with 95% $O_2$ and 5% $CO_2$. Detrusor smooth muscle (integrated detrusor smooth muscle bundles) from the anterior wall of the bladder body was dissected in a longitudinal direction. Any visible fat, connective tissue, vessels, and mucosa were removed. Then smooth muscle was cut into strips measuring 1.5 mm in width and 5 mm in length for the tension experiments.

*Tension measurement*

The detrusor strips were mounted in an organ bath. Isometric tension was continuously measured with a force transducer (FDT10-A; Commat, Ltd., Ankara, Turkey), connected to a computer-based data acquisition system (TDA 97, Commat, Ltd.). A resting tension of 1 g was applied and then the strips were allowed to equilibrate for 1 h before any agonists were added. The preparations were washed with PSS every 15 min during the equilibration period. The effects of carbachol ($10^{-7} – 10^{-4}$ M) and isoproterenol ($10^{-6} – 10^{-3}$ M) in a cumulative manner on basal tone tissues were recorded. Cumulative concentration-response curves were constructed in a stepwise manner after the responses to the previous concentration had reached a plateau. After each concentration-response curve, the preparation was washed 3 times with PSS and the next concentration-response was constructed after 45 min of equilibration. After obtaining the isoproterenol concentration-response curve, papaverine (100 $\mu$M) was added to the bath to elicit maximal relaxation at the end of the experiments and the results were expressed as a percentage of these maximum responses. Carbachol responses were also expressed as a percentage of their maximum responses.

*Analysis of results*

At the end of each experiment, detrusor smooth muscles were detached from the recording set-up, blotted, and weighed. The contractile and relaxant responses were expressed as milligrams (mg) of tension developed per mg of tissue-wet weight. All values were expressed as means ± S.E.M. and "n" indicated the number of animal preparations. Sensitivity was expressed as $pD_2$ ($-\log EC_{50}$). Smooth muscle contractility and relaxation was evaluated as the maximally developed tension and relaxant effect per unit tissue

weight ($E_{max}$). Statistical analysis of the results was performed using the analysis of variance and Student's *t*-test. *P* values lower than 0.05 were considered statistically significant.

## Results

Two weeks after partial obstruction of the urethral outflow led to a significant increase in bladder weight (350 ± 21 mg), when compared to the sham-operated rats (180 ± 13 mg). There was no significant difference in the baseline tension of the spontaneous activity between rat detrusor smooth muscles from sham-operated and partially obstructed bladder ($P>0.05$, n = 12).

*Effect of carbachol on detrusor smooth muscle preparations*

Carbachol caused concentration-dependent contractile responses in rat detrusor smooth muscles from the sham-operated and partially obstructed bladder (Fig. 1). The contractile responses to carbachol in rat detrusor smooth muscles from obstructed bladder were significantly enhanced when compared to sham-operated animals, but not in rat detrusor smooth muscles from partially obstructed bladder of rats treated with doxazosin. The $E_{max}$ values and the $pD_2$ of the carbachol are shown in Table 1.

*Effect of isoproterenol on detrusor smooth muscle preparations*

Isoproterenol produced concentration-dependent relaxation responses in the detrusor smooth muscles from all groups (Fig. 2). Concentration-response curves for isoproterenol showed a shift to the left in rat detrusor smooth muscles from obstructed bladder when compared to the results obtained in the other groups. The effects of isoproterenol were reversed by doxazosin treatment in rat detrusor smooth muscles from obstructed bladder. The $E_{max}$ values and the $pD_2$ of the isoproterenol are shown in Table 1.

*Effect of KCl on detrusor smooth muscle preparations*

KCl produced contractile responses in rat detrusor smooth muscle preparations from all groups. The contractile responses to KCl were not statistically different among groups. The $E_{max}$ values of the KCl are shown in Table 1.

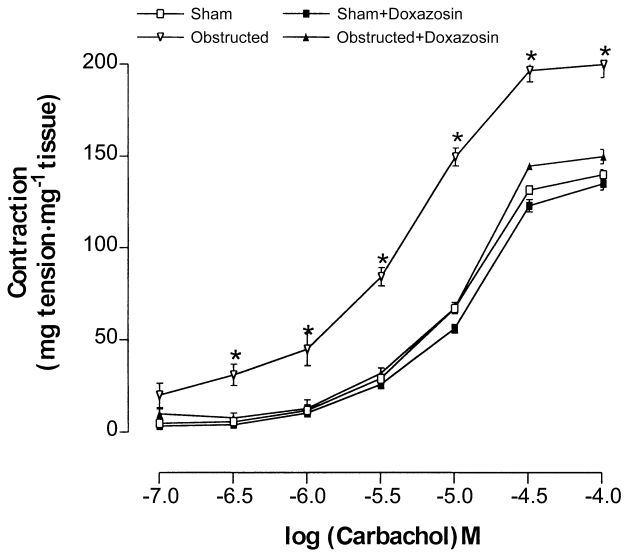


**Fig. 1.** The contractile responses to carbachol in detrusor smooth muscle preparations from sham-operated, sham treated with doxazosin, obstructed, and obstructed treated with doxazosin rats. (Data are reported as the mean ± S.E.M., n = 12; *statistically different from sham, sham + doxazosin, obst + doxazosin, $P<0.05$).

**Table 1.** The $pD_2$ ($\mu$M) and $E_{max}$ (% inhibition) values of the carbachol, isoproterenol, and KCl

| | | Carbachol | Isoproterenol | KCl |
|---|---|---|---|---|
| Sham-operated | $pD_2$ | 4.8 ± 0.09 | 5.2 ± 10.08 | |
| | $E_{max}$ | 140 ± 2.9 | 90.0 ± 1.2 | 185 ± 6.3 |
| Sham + Doxazosin | $pD_2$ | 4.8 ± 0.09 | 5.2 ± 10.08 | |
| | $E_{max}$ | 135 ± 3.4 | 82.0 ± 3.1 | 180 ± 8.3 |
| Obstructed | $pD_2$ | 5.2 ± 0.07* | 5.08 ± 0.14* | |
| | $E_{max}$ | 200 ± 7.0* | 110.3 ± 1.3* | 171 ± 5.3 |
| Obst. + Doxazosin | $pD_2$ | 4.9 ± 0.07 | 5.0 ± 0.11 | |
| | $E_{max}$ | 170 ± 4.0 | 84.2 ± 4 | 176 ± 4.1 |

Values are means ± S.E.M., n = 12; *statistically different from sham-operated, sham + doxazosin, obst. + doxazosin ($P<0.05$).

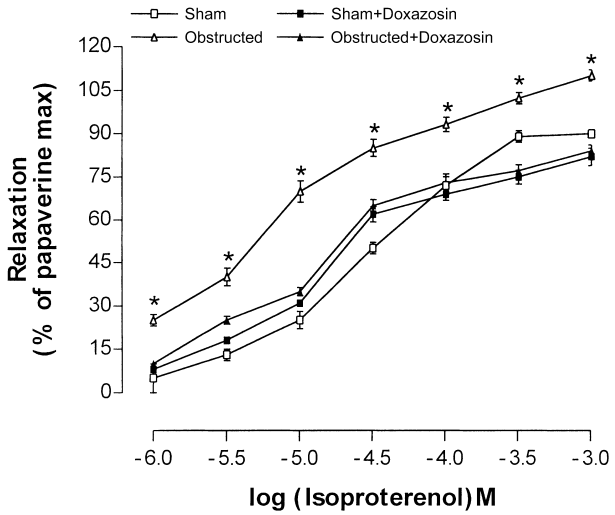


**Fig. 2.** The relaxation responses to isoproterenol in detrusor smooth muscle preparations from sham-operated, sham treated with doxazosin, obstructed, and obstructed treated with doxazosin rats. (Data are reported as the mean ± S.E.M., n = 12; *statistically different from sham, sham + doxazosin, obst + doxazosin, $P<0.05$).

## Discussion

The present study showed that 2 weeks after surgery, rat bladder weights increased significantly in the obstructed groups. Since the obstructed urinary bladder is characterized by detrusor smooth muscle hypertrophy, these increases seemed to be related to increment of the bladder mass (7 – 9).

Generally, results from studies using animal models of bladder outlet obstruction report rapid and marked morphological and functional changes in the detrusor muscle, similar to those reported in human clinical studies (10, 11). Results of research studies on these animal models show that bladder outlet obstruction disrupts bladder blood flow and that the resulting ischemia/hypoxia of this tissue trigger bladder hypertrophy as well as the subsequent dysfunctions that develop in this tissue (12 – 14). However, how this dysfunction of the detrusor smooth muscle occurs is still not fully known.

In patients, symptoms associated with bladder outlet obstruction can be divided into the 2 general categories of obstructive (hesitancy, poor stream, prolonged urination, and sensation of incomplete emptying) and irritative (frequency, urgency, nocturia, and unstable bladder contractions). In these patients, irritative symptoms may persist even after removal of obstruction, as documented by subsequent normalization of urine flow rates. This observation support that patients with associated bladder outlet obstruction have detrusor overactivity, which often leads to uninhibited detrusor contractions (15).

In animal models, outlet obstruction causes an increase in bladder mass and contractile dysfunction in vivo and in vitro (16, 17). Previous studies have shown modified responses of detrusor strip to cholinergic agonists. For example, an apparent decrease in the responses of obstructed rat bladder to bethanechol has been reported (18). Another study showed that the force of contraction from obstructed guinea pigs was less than that observed in control guinea pigs (19). Also, it has been reported that carbachol responses decreased in rabbit bladder partially obstructed for up to 70 days (20). In contrast to these studies, it has also been shown that sensitivity to carbachol may increase in the smooth muscle strips from obstructed pig bladders (21 – 24). Previously, a twofold increase in muscarinic receptor binding sites at the level of obstructed detrusor muscle has been shown (22). In another study, a partial outlet obstruction in the rat resulted in a progressive increase in bladder mass; an increase in nicturition frequency; increases in the in vitro contractile response to field stimulation, bethanechol, methoxamine, and KCl; and increases in bladder DNA content and $^{3}$H-thymidine incorporation (23). These latter authors have found that smooth muscle from the rabbit obstructed bladders showed enhanced sensitivity and higher levels of force to both KCl and carbachol during cumulative additions (24). The discrepancies of response to carbachol may be due to duration of the bladder obstruction and to using different animal species. Also, in the present study, we found that carbachol responses increased in rat obstructed bladder when compared to sham-operated rats and these increases returned to normal in animals treated with doxazosin. These findings points out that increased contraction responses of rat obstructed bladders may be improved through $\alpha$-blocker treatment. Since patients with associated bladder outlet obstruction have detrusor overactivity, this effect of doxazocin may be helpful.

In the last years, there is evidence that the sympathetic nervous system and $\alpha$1ARs have an important role in the treatment of bladder outlet obstruction (1, 25). Because of the usefulness of $\alpha$1AR antagonists for treating symptoms associated with bladder outlet obstruction and the recent suggestion that $\alpha$1AR antagonist may relieve obstruction via the relaxation of prostate-bladder neck smooth muscle (25 – 29), we examined the effect of doxazosin on cholinergic and adrenergic responses in detrusor smooth muscle preparations from obstructed bladder. It has been demonstrated that $\beta$-ARs of rat detrusor smooth muscle are considered to be mixed populations consisting of

three subtypes that play an important role in relaxing smooth muscle in response to catecholamines (30). It has been shown that the muscle strips from rabbit obstructed bladder showed reduced inhibitory responses to $\beta$-adrenergic stimulation with isoprenaline (31). In another study, a relaxant effect in response to isoproterenol 2 weeks after obstruction was obtained, but this was not so 6 weeks after obstruction. These findings suggest that the duration of obstruction is important and may change the response to the same agonist (32). However, there is a study indicating a remarkable increase in bladder $\alpha$1d-adrenoceptor M RNA and protein expression after 6 weeks of obstruction and resultant detrusor hypertrophy (9). In our study, we also found that relaxation responses to isoproterenol increased in 2-week rat obstructed bladders. These increased relaxation responses were decreased by the treatment with the $\alpha$-blocker doxazosin.

In a previous study, it was found that detrusor smooth muscle strips from rat obstructed bladder showed no significant differences in contraction response to noncumulative addition of KCl (24). Also, we found that the contraction produced with KCl was not significantly different among groups. These findings demonstrate that there is no impairment of the contraction machinery in smooth muscles from rat obstructed bladder.

In conclusion, in the present study, we showed that responses to cumulatively applied carbachol and isoproterenol were shifted to the left in detrusor smooth muscles of rat obstructed bladder. Increased responses to carbachol and isoproterenol may be due to increased receptor number or postjunctional supersensitivity. Moreover, since these responses were reversed to normal by doxazosin treatment, we suggest that the effects of doxazosin on $\alpha$1ARs are involved.

## Acknowledgement

This study was supported by research from Akdeniz University Research Foundation.

## References

1 Levin RM, Shofer FS, Wein AJ. Cholinergic, adrenergic and purinergic response of sequential strips of rabbit urinary bladder. J Pharmacol Exp Ther. 1980;212:536–540.
2 Michelotti GA, Price DT, Schwinn DA. $\alpha$1-Adrenergic receptor regulation: basic science and clinical implications. Pharmacol Ther. 2000;83:281–309.
3 Yang SS, Wang CC, Hsies CH, Chen YT. Alpha1-adrenergic blocker in young men with primary bladder neck obstruction. J Urol. 2002;168:571–574.
4 Perepanova TS, Kamalov AA, Siniukhin VN, Gorokhnov AV, Khazan PL, Orlova EV. Doksazozin" cardura" in acute urinary retention caused by benign prostatic hyperplasia. Urologia. 2001;3:18–20.
5 Kaplan SA, Stifelman M, Avillo C, Reis RB, Te AE. Detrusor contraction duration may predict response to alpha-blocker therapy for lower urinary tract symptoms. Eur Urol. 2000;37: 314–317.
6 Lepor H, Auerbach S, Puras-Baez A, Narayan P, Soloway M, Lowe F, et al. A randomized, placebo-controlled multicenter study of the efficacy and safety of terazosin in the treatment of benign prostatic hyperplasia. J Urol. 1992;148:1467–1474.
7 Saito M, Longhurst PA, Tammela TL, Wein AJ, Levin RM. Effect of partial outlet obstruction of the rat urinary bladder on micturition characteristics, DNA synthesis and the contractile response to field stimulation and pharmacological agents. J Urol. 1993;150:1045–1051.
8 Igawa Y, Mattiasson A, Andersson KE. Micturition and premicturition contractions in unanaesthetized rats with bladder outlet obstruction. J Urol. 1994;151:244–249.
9 Hampel C, Dolber PC, Smith MP, Savic SL, Thuroff JW, Thor KB, et al. Modulation of bladder alpha1-adrenergic receptor subtype expression by bladder outlet obstruction. J Urol. 2002;167:1513–1521.
10 Jorgensen TM, Djurhuus JC, Jorgensen HS, Sorensen SS. Experimental bladder hyperreflexia in pigs. Urol Res. 1983;11: 239–240.
11 Mattiasson A, Uvelius B. Changes in contractile properties in hypertrophic rat urinary bladder. J Urol. 1982;128:1340–1342.
12 Levin RM, Brading AF, Mills IW, Longhust PA. Prostatic disease. St. Louis: WB Saunders Co; 1999.
13 Levin RM, Memberg W, Ruggier MR, Wein AJ. Functional effects of in vitro obstruction on the rabbit urinary bladder. J Urol. 1986;135:847–851.
14 Saito M, Yokoi K, Ohmura M, Kondo A. Effects of partial outflow obstruction on bladder contractility and blood flow to the detrusor: comparison between mild and severe obstruction. Urol Int. 1997;59:226–230.
15 Seaman EK, Jacobs BZ, Biaivas JG, Kaplan SA. Persistence or recurrence of symptoms after transurethral resection of the prostate: a urodynamic assessment. J Urol. 1994;152:935–937.
16 Levin RM, Brading AF, Mills IW, Lonhust PA. Experimental models of bladder obstruction. In: Lepor H, editor. Prostatic disease. Philadelphia: WB Saunder; 2000. p. 169–196.
17 Kato K, Monson FC, Longhurst PA, Wein AJ, Haugaard N, Levin RM, et al. The functional effects of long-term outlet obstruction on the rabbit urinary bladder. J Urol. 1990;143:600–609.
18 Levin RM, High J, Wein AJ. The effect of short term obstruction on urinary bladder functions in the rabbit. J Urol. 1984;132:789–791.
19 Williams JH, Turner WH, Sainsbury GM, Brading AF. Experimental model of bladder outflow tract obstruction in the guinea-pig. Br J Urol. 1993;71:543–554.
20 Gosling JA, Kung LS, Dixon JS, Horan P, Whitbeck C, Levin RM. Correlation between the structure and function of the rabbit urinary bladder following partial outlet obstruction. J Urol. 2000;163:1349–1356.
21 Sibley GNA. The response of the bladder to lower urinary tract obstruction. DM Theses, 1984; Oxford.
22 Beavington TE, Morrison J, Robertson AS, Neal DE. Muscarinic

receptors and beta-adrenoceptor binding sites in the normal and unstable bladder. Br J Pharmacol. 1993;110:116–120.

23 Saito M, Longhurst PA, Tammela TL, Wein AJ, Levin RM. Effects of partial outlet obstruction of the rat urinary bladder on micturition characteristics, DNA synthesis and the contractile response to field stimulation and pharmacological agents. J Urol. 1993;150:1045–1051.

24 Su X, Stein R, Stanton MC, Zderic S, Moreland RS. Effect of partial outlet obstruction on rabbit urinary bladder smooth muscle function. Am J Physiol Renal Physiol. 2003;284:F644–F652.

25 Schwinn DA, Michelotti GA. $\alpha_1$-Adrenergic receptors in the lower urinary tract and vascular bed: potential role for the $\alpha$1d subtype in filling symptoms and effects of aging on vascular expression. BJU Int. 2000;85:6–11.

26 Forray C, Bard JA, Wetzel JM, Chiu G, Shapiro E, Tang R, et al. The $\alpha$1-adrenergic receptor that mediates smooth muscle contraction in human prostate has the pharmacological properties of the cloned $\alpha$1c subtype. Mol Pharmacol. 1994; 45:703–708.

27 Blue DJ, Grino P, Jung D. Evaluation of Ro 70-0004, a selective $\alpha$1a-adrenoceptor antagonist, in men with bening prostatic hyperplasia. Presented at International Consultation on BPH, Paris, France, June 25 – 28, 2000.

28 Matyus P, Horvath K. $\alpha$-Adrenergic approach in the medical management of benign prostatic hyperplasia. Med Res Rev. 1997;17:523–535.

29 Michel MC, Mehlburger L, Schumacher H, Bressel HU, Goepel M. Effects of diabetes on lower urinary tract symptoms in patients with benign prostatic hyperplasia. J Urol. 2000;163: 1725–1729.

30 Seguchi H, Nishimura J, Zhou Y, Niiro N, Kumazawa J, Kanaide H. Expression of beta3-adrenoceptors in rat detrusor smooth muscle. J Urol. 1998;159:2197–2201.

31 Harrison SC, Ferguson DR, Doyle PT. Effect of bladder outflow obstruction on the innervation of the rabbit urinary bladder. Br J Urol. 1990;66:372–379.

32 Moore CK, Levendusky M, Longhurst PA. Relationship of mass of obstructed rat bladders and responsiveness to adrenergic stimulation. J Urol. 2002;168:1621–1625.