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Denervation and outlet obstruction induce a net synthesis of contractile and cytoskeletal proteins in the urinary bladder of the male rat

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Abstract The concentrations of the contractile proteins actin and myosin and the cytoskeletal protein desmin were determined in urinary bladders from normal rats, and from rats with bladder outlet obstruction or denervation. Ten days of obstruction or total denervation by bilateral removal of the pelvic ganglia resulted in an almost fourfold increase in bladder weight. Actin and myosin concentrations did not change significantly. The total amount of actin was 1624 ± 235 μg in the control bladder. In the obstructed and denervated bladders it increased significantly to 6277 ± 648 μg and 7671 ± 835 μg, respectively. The desmin/actin ratio was 0.237 ± 0.012 in the control bladders, and increased significantly to 0.369 ± 0.015 in the obstructed and 0.343 ± 0.022 in the denervated bladders. Partial denervation by removal of the pelvic ganglion on one side only increased bladder weight by 52%, but did not increase the desmin/actin ratio. The content of actin in such bladders increased by 82%. Both obstruction (which increases the functional load of the detrusor muscle cells) and denervation (which produces bladder paralysis) are known to induce hypertrophy of the detrusor smooth muscle cells. The study shows that the desmin/actin ratio and the total amount of contractile proteins increase in response to the hypertrophy as such, and not to the work performed by the smooth muscle cells, and that the nerves have no trophic influence on the growth response. Also, even a limited lesion of the bladder innervation is associated with growth and a net increase in the amount of contractile proteins.

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Introduction

Partial infravesical outlet obstruction increases both micturition time and micturition pressure [13]. The detrusor smooth muscle cells adapt to this increased functional load by a considerable degree of hypertrophy [9], which is evident by the increased cross-sectional area of the cells. In the rat bladder [15] the hypertrophy of the smooth muscle cells is accompanied by a net synthesis of the contractile proteins actin and myosin with an increase in total bladder content of these proteins. The function of the intermediate filaments is not known with certainty [11], but they are considered to constitute a part of the cellular skeleton in smooth muscle [18]. The intermediate filament protein desmin increases more than that of the contractile proteins, resulting in an increased desmin/actin ratio [15, 16]. The reason for this is not known but it might be a response to the increased functional load or to the increased volume of the smooth muscle cells. The amounts of intermediate filaments [3, 8] and desmin [14] have been shown to increase in other types of hypertrophic smooth muscle.

As it is not known whether the increased desmin/actin ratio and the increased total bladder content of actin and myosin reflect an increased workload on the detrusor muscle cells or merely reflects their increased size, we have chosen to compare obstructed bladders and totally denervated bladders in these respects. In both groups the cross-sectional areas of the detrusor muscle cells are known to increase considerably [5, 9, 19]. In both types of bladder growth, distension of the bladder by the urine is present. In the obstructed bladders the detrusor muscle might, in addition, be subjected to an increased workload, whereas

the muscle in the denervated bladders is not active. The obstructed bladders might also be influenced by trophic stimuli from the bladder nerves. A study of the effects of a partial denervation of the bladder on actin and myosin content and desmin/actin ratio is included in order to elucidate how sensitive the bladder content of contractile and intermediate filament proteins is to more limited disturbances of its function.

Methods

A total number of 31 male rats of the Sprague-Dawley strain weighing about 250 g were used.

Surgery and animal care

The rats were anesthetized by ketamine (Ketalar) 100 mg/kg and xylazine (Rompun) 15 mg/kg, given i.m., and the bladder and prostate were visualized through a lower midline incision. One group of the animals then underwent a partial obstruction of the urethra between the prostate and the bladder using 3/0 silk tied around a metal rod (diameter 1 mm), which was then removed. Another group underwent bilateral removal of the pelvic ganglia (for anatomical description see ref. [1]), resulting in a total denervation of the bladder. In a third group only one ganglion was removed. Sham-operated animals served as controls.

Bilaterally ganglionectomized rats are, in contrast to obstructed and unilaterally ganglionectomized rats, unable to pass urine. The bladders were therefore emptied manually every day.

Ten days after the operation the animals were killed by cervical fracture and the bladder was dissected out and weighed. All bladder material from the obstructed or bilaterally ganglionectomized bladders was weighed and then frozen in liquid nitrogen and stored at −80 °C until the analysis described below. The unilaterally ganglioneztomized bladders were cut open along the ventral surface and mounted in a Petri dish. A punch biopsy (diameter 4 mm) was then taken from the left- and rightmost parts of the bladder in the equatorial plane. The biopsies were then used for the analysis below.

Extraction of proteins and SDS-polyacrylamide electrophoresis

The bladder tissue was thawed and homogenized in a sodium dodecyl sulfate (SDS) buffer (50 µl/mg wet weight) until no particulate matter remained. The homogenate was then boiled for 2 min and stored at −20 °C prior to analysis with quantitative electrophoresis. The SDS buffer contained 25 mM TRIS (hydroxymethyl) aminomethane hydrochloride (pH 6.8), 2% SDS (sodium lauryl sulfate), 5% mercaptoethanol and 10% glycerol. The SDS polyacrylamide gel electrophoresis (SDS-PAGE) was performed essentially as described by Laemmli [12] using 8% polyacrylamide gels in a BioRad minigel system (Bio-Rad, Richmond, Calif., USA). We have previously shown that the myosin heavy-chain bands in extracts from rat bladder contain only a small fraction of non-muscle myosin and that the intermediate filament band comprises mainly desmin [15]. Four different volumes of sample and of skeletal muscle actin with a known concentration were run in parallel on each gel. The gels were stained with Coomassie blue and scanned using a GS-300 densitometer (Hoefer Scientific Instruments, San Francisco, Calif., USA). The areas under the myosin heavy chain, the actin, and the desmin bands were calculated. From the gel scans, the sample (and tissue) concentration of actin and the ratios actin/myosin and desmin/actin were evaluated.

Statistics

Values are given as means ± SE, with number of animals indicated. Comparisons between controls and partially denervated bladders were made using Student's *t*-test for unpaired data. Comparisons between the right and left side in the unilaterally ganglionectomized bladders, and their controls, were made using Student's *t*-test for paired data. Simultaneous comparisons between obstructed bladders, the bilaterally ganglionectomized bladders, and their controls were made using the Bonferroni test. *, **, and *** denote global probability levels of $P < 0.05$, 0.01, and 0.001, respectively.

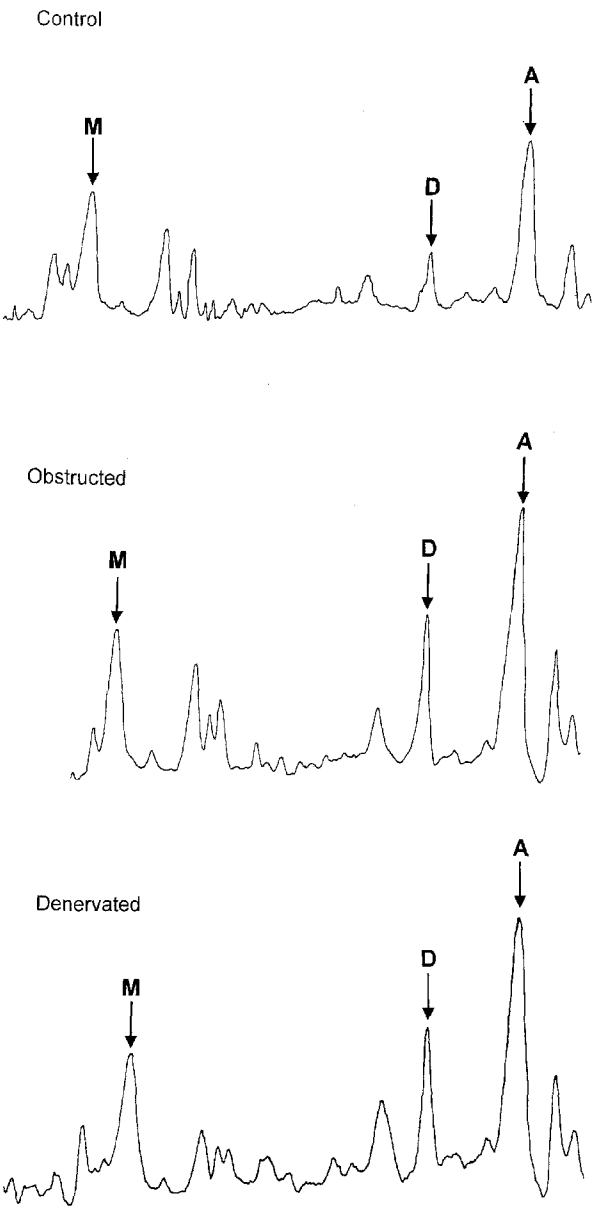

Fig. 1 Typical densitometric gel scans of SDS-PAGE gels from a control bladder (*upper panel*), an obstructed bladder (*middle panel*), and a totally denervated bladder (*lower panel*). The myosin (*M*), desmin (*D*), and actin (*A*) bands are indicated. Note the increase in desmin relative to actin in the obstructed and denervated bladders

Results

Comparison between obstructed and completely denervated bladders

The bladder weight increased almost fourfold after 10 days of partial outlet obstruction or bilateral ganglionectomy (Fig. 2A). Figure 1 shows typical densitometric gel scans of SDS-PAGE gels from the three groups studied. Figure 2 summarizes the results from the whole material. The concentration of actin and the actin/myosin ratio were not significantly affected by the bladder growth in either situation (Fig. 2B, C). The desmin/actin ratio increased significantly in both groups. There was no significant difference between denervated and obstructed bladders with regard to weight, actin or myosin concentration or desmin/actin ratio.

The total amount of actin in the control bladders (calculated from bladder weight and actin concentration) was 1624 ± 235 µg ($n = 10$). After 10 days of partial obstruction or complete denervation the corresponding values were 6277 ± 648 µg ($n = 7$) and 7671 ± 835 µg ($n = 7$), respectively. Both increases are significant ($P < 0.001$) according to the Bonferroni test. A considerable net synthesis of actin has thus been induced. As we have not used any myosin standard, the absolute amount of myosin cannot be determined. The unchanged actin/myosin ratios show, however, that the net synthesis of myosin is similar to that of actin.

Partially denervated bladders

Partial denervation of the urinary bladder induced a significant increase by about 50% in bladder weight (Fig. 3A). This increase in weight was, however, not associated with any significant changes in actin/myosin (Fig. 3C) or desmin/actin (Fig. 3D) ratios in any of the bladder halves. There was a small but significant increase in actin concentration in the denervated side of the bladder compared with the contralateral (Fig. 3B).

The amounts of actin (calculated from mean actin concentration in the biopsies from the right and left side, and the bladder weight) in the control bladders was 976 ± 88 µg ($n = 6$). The main reason for the lower values in this group than in the controls in the previous section is their lower bladder weights (c.f. Figs. 2A and 3A). After unilateral ganglionectomy the actin content per bladder increased to 1778 ± 214 ($n = 8$) µg. This increase was significant ($P < 0.01$) according to Student's t-test for unpaired data. The unchanged

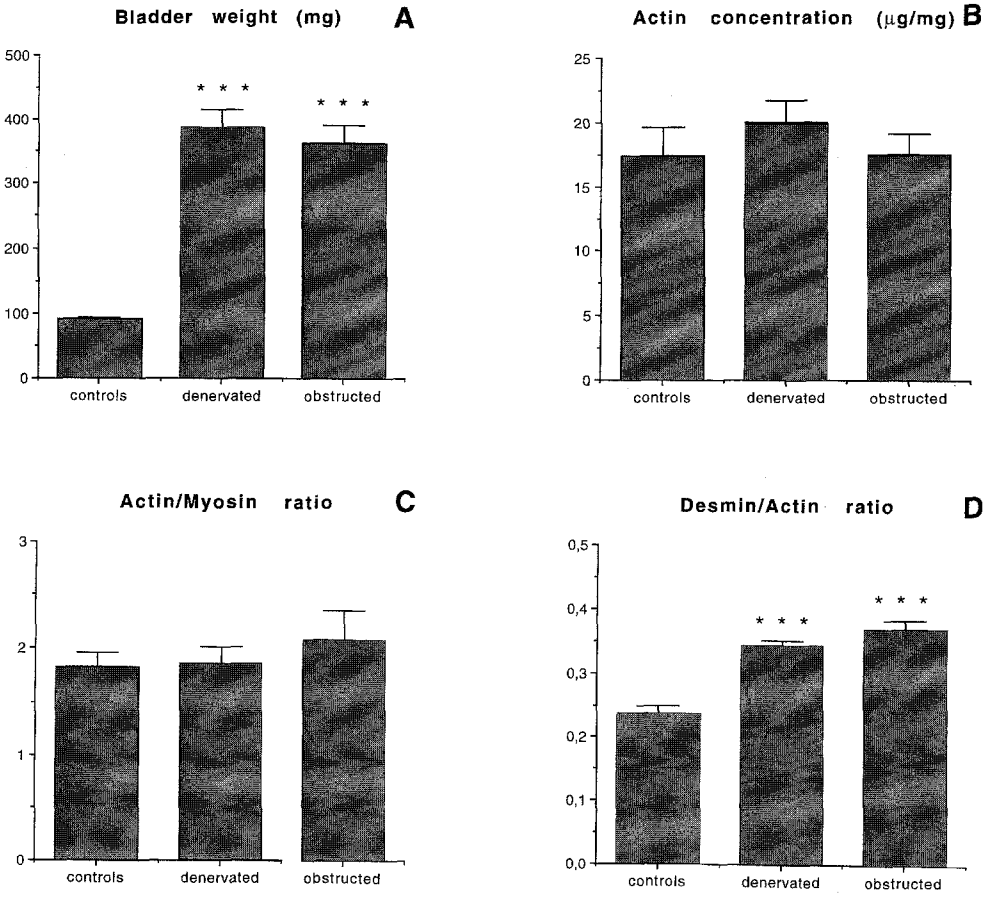

Fig. 2 Bladder weight (**A**), actin concentration (**B**), actin/myosin (**C**), and desmin/actin ratios (**D**) in bladders from ten control rats, seven rats with bilateral removal of the pelvic ganglia, and seven rats with infravesical outlet obstruction. Bladder denervation and outlet obstruction increased significantly the bladder weight and the desmin/actin ratio. There was no significant difference between the two operated groups. Statistical comparison between the three groups was made using the Bonferroni test

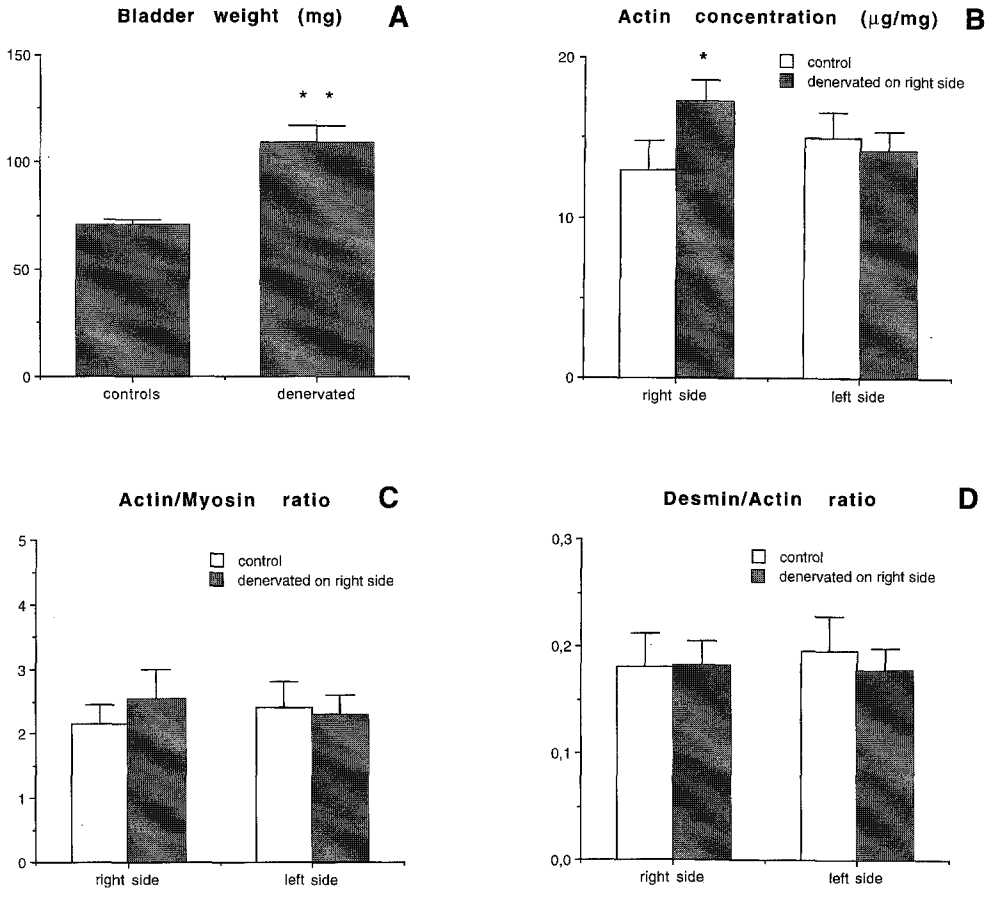

Fig. 3 A shows bladder weight in six control bladders and eight bladders from rats with removal of the right pelvic ganglion 10 days previously. The 50% increase in bladder weight in the operated group is significant. The actin concentration, actin/myosin, and desmin/actin ratios in punch biopsies from the right and left side of the bladders are given in **B–D** respectively. Statistical comparison between right and left side was made using Student's *t*-test for paired data. There was a slight but significant increase in actin concentration in the right (denervated) half of the operated group

actin/myosin ratio in the unilaterally ganglionectomized group indicates a similar increase in myosin content per bladder.

Discussion

Both partial infravesical obstruction and total denervation of the urinary bladder induce bladder growth. The obstructed rat bladders increase about fourfold in weight after 10 days, and about tenfold after 7 weeks [15]. At corresponding times the denervated bladders weigh about 4 and 6 times more than normal [19].

To some extent the increased weight is due to an increased amount of extracellular proteins. The total amount of collagen per bladder is significantly higher after 10 days of obstruction [20] or denervation [19]. Most of the weight gain seems, however, to be due to hypertrophy of the detrusor smooth muscle cells.

In control rat detrusor smooth muscle cells the cross-sectional area at 0.7 ml of bladder filling is 6–8 μm^2 [5, 19, 21]. This increases to about 39 μm^2 after 6 weeks of obstruction [21]. Total denervation increases the cross-sectional area to 17 μm^2 after 10 days [5] and to about 24 μm^2 after 6 weeks [19]. There is thus a considerable degree of hypertrophy in both types of growth.

The denervated bladder is paralyzed and cannot void actively. The bladders rapidly become overdistended, and we have noted that overflow incontinence develops. In order to avoid excessive overdistension of the bladder wall, we emptied the bladder manually every 24 h. It is reasonable to assume that the stimulus for detrusor cell hypertrophy is the stretch similar to that proposed for the pregnant uterus [4].

The situation is more complex in the obstructed bladder. The obstruction produces a considerably higher micturition pressure and a prolonged micturition. This imposes an increased functional load on the detrusor muscle cells, which is one probable stimulus for compensatory hypertrophy (if the obstruction is removed the cross-sectional areas decrease to almost normal values; see ref. [10]). Also, the obstructed bladder develops a residual urine [13], which might add to the growth stimulus by the stretch of the smooth muscle cells. It could also be possible that the bladder nerves exert a trophic influence on the smooth muscle cells.

In principle, three types of filaments exist in smooth muscle cells. The interaction of thick (myosin) and thin (actin) filaments converts chemical energy to active

force and/or shortening. Also, intermediate filaments consisting mainly of desmin are found between the actin and myosin filaments. The function of the intermediate filaments is not known with certainty [11], but they are considered to constitute a part of the cellular skeleton in smooth muscle [18].

The desmin/actin ratio has been shown to increase in hypertrophic smooth muscle [14] from rat portal vein. By electron microscopy an increased amount of intermediate filaments has been demonstrated in obstructed rabbit portal vein [3] and rat intestine [7, 8]. Common to all three studies is that the functional load of the smooth muscle cells has increased by the obstruction. It could be that the increased amount of intermediate filaments reflects a need for a more developed and sturdy cytoskeleton in the hypertrophic cells with their larger volumes.

The present study is to our knowledge the first where two different stimuli of hypertrophy are compared with regard to content of actin, myosin and desmin in a smooth muscle organ.

The major part of the myosin molecule, the myosin heavy chain, exists in two isoforms, SM1 and SM2, whereas three isoforms (α, β and γ) of actin are found. Infravesical obstruction in the rat [15, 16] and rabbit [17] leads to a decreased relative amount of SM2, whereas in bladder biopsies from patients with prostatism a slight increase has been reported [16]. In the rat [15] obstruction leads to a relative increase in α-, and a decrease in γ-actin, whereas in the human α-actin increases and β-actin decreases [16]. The isoform distribution of the newly synthesisized contractile proteins is thus different from the normal pattern although the physiological relevance of this is unknown [17]. We therefore chose in the present study to simplify the analysis by not determining actin and myosin isoforms.

Both types of stimuli of bladder growth increased the bladder weight about fourfold and induced a considerable net synthesis of actin and myosin. There was, for the same proteins, no significant difference between the two groups. Also, the increase in desmin/actin ratio was similar for both groups. This shows that the presence or absence of nerves did not influence the growth response of the bladder as a whole or the content of contractile or cytoskeletal proteins.

We have previously [15, 16] found that the actin/myosin ratio increased in the obstructed bladders. In the present study we did not find any significant difference in this ratio between the control, denervated or obstructed bladders, although a tendency to an increase was found in the latter group.

Although the disturbance in bladder function after removal of one pelvic ganglion is limited and consists only of increased micturition volume and intervals [2], the growth stimulus was sufficient to induce a 50% increase in bladder weight. It has previously been reported that, although the weight increases in both bladder halves, the increase is more pronounced on the bladder half ipsilateral to the ganglionectomy [6]. We found a significant increase in total bladder content of both actin and myosin. There was a significant increase in actin concentration on the side of the denervation, but we do not know whether that has any functional relevance. The increase could simply be due to different relative proportions in smooth muscle/non-muscle tissue between the different groups of bladders. The desmin/actin ratio did not change following unilateral ganglionectomy, indicating that a 50% increase in bladder weight is too limited.

In summary, both infravesical obstruction and total denervation induced a fourfold increase in bladder weight associated with a similar net synthesis of actin and myosin, and a similar increase in the desmin/actin ratio. There is thus no difference in these respects between the hypertrophy induced by stretch or increased functional load of the detrusor smooth muscle cells.

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References

1. Baljet B, Drukker J (1979) The extrinsic innervation of the abdominal organs in the female rat. Acta Anat 104:243
2. Berggren T, Gabella G, Malmgren A, Uvelius B (1993) Effects of unilateral pelvic ganglionectomy on urinary bladder function in the male rat. Scand J Urol Nephrol 27:181
3. Berner PF, Somlyo AV, Somlyo AP (1981) Hypertrophy-induced increase of intermediate filaments in vascular smooth muscle. J Cell Biol 88:96
4. Cullen BM, Harkness RD (1968) Collagen formation and changes in cell population in the rat uterus after distension with wax. Q J Exp Physiol 53:33
5. Ekström J, Uvelius B (1983) Changes in length and volume of smooth muscle cells of the hypertrophied rat urinary bladder. Acta Physiol Scand 118:305
6. Ekström J, Malmberg L, Öberg S (1986) Unilateral denervation of the rat urinary bladder and reinnervation: a predominance for ipsilateral changes. Acta Physiol Scand 127:223
7. Gabella G (1975) Hypertrophy of intestinal smooth muscle. Cell Tiss Res 163:199
8. Gabella G (1979) Hypertrophic smooth muscle. IV. Myofilaments, intermediate filaments and some mechanical properties. Cell Tiss Res 201:277
9. Gabella G, Uvelius B (1990) Urinary bladder of the rat: fine structure of normal and hypertrophic musculature. Cell Tiss Res 262:67
10. Gabella G, Uvelius (1994) Reversal of muscle hypertrophy in the rat urinary bladder after removal of urethral obstruction. Cell Tiss Res 277:333
11. Geiger B (1987) Intermediate filaments: looking for a future. Nature 329:392
12. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of the bacteriophage T_4. Nature 227:680
13. Malmgren A, Sjögren C, Uvelius B, Mattiasson A, Andersson K-E, Andersson PO (1987) Cystometrical evaluation of bladder instability in rats with infravesical outflow obstruction. J Urol 137:1291

14. Malmqvist U, Arner A (1990) Isoform distribution and tissue content of contractile and cytoskeletal proteins in the hypertrophied smooth muscle from rat portal vein. Circ Res 66:832
15. Malmqvist U, Arner A, Uvelius B (1991) Contractile and cytoskeletal proteins in smooth muscle during hypertrophy and its reversal. Am J Physiol 260:C1085
16. Malmqvist U, Arner A, Uvelius B (1991) Cytoskeletal and contractile proteins in detrusor smooth muscle from patients with bladder outlet obstruction. Scand J Urol Nephrol 25:261
17. Samuel M, Kim Y, Horiuchi K, Levin RM, Chacko S (1992) Smooth muscle myosin isoform distribution and myosin ATPase in hypertrophied urinary bladder. Biochem Int 26:645
18. Small JV, Sobieszek A (1980) The contractile apparatus of smooth muscle. Int Rev Cytol 64:241
19. Uvelius B, Mattiasson A (1986) Detrusor collagen content in the denervated rat urinary bladder. J Urol 136:1110
20. Uvelius B, Mattiasson A (1984) Collagen content in the rat urinary bladder subjected to infravesical outflow obstruction. J Urol 132:587
21. Uvelius B, Persson L, Mattiasson A (1984) Smooth muscle cell hypertrophy and hyperplasia in the rat detrusor after short-time infravesical outflow obstruction. J Urol 131:173