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Effects of Partial Bladder Outlet Obstruction and Its Relief on Types I and III Collagen and Detrusor Contractility in the Rat

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Bladder outlet obstruction induces a rapid hypertrophy characterized by increased bladder mass and collagen deposition. An increase in collagen is likely to reduce the contractility and compliance of bladder wall. This study was undertaken to investigate the effects of partial bladder outlet obstruction and its relief on types I and III collagen, and the relationship between detrusor contractility and collagen types. A total of 40 female rats was used for experiment and divided into one control, one obstruction, and three recovery groups. The contractility to field stimulation was recorded; total collagen and collagen concentration were quantified. The localization of types I and III collagen and the expression of pro-α1(I) and α1(III) collagen mRNA were determined by immunohistochemical staining and Northern blot hybridization, respectively. Contractile response to field stimulation was reduced after obstruction and recovered following relief. The total amount of collagen increased after obstruction and decreased after relief; however, collagen concentration decreased after obstruction and increased following relief. Contractility correlated negatively with total collagen but positively with collagen concentration. The protein deposition of types I and III collagen was localized in lamina propria and muscle bundles in all groups. The expression of types I and III collagen gene was up regulated after obstruction, but down regulated after relief. Negative correlation between contractility and gene expressions of collagen types was significant. These data suggest that the change in localization and quantity of collagen types leads to morphologic changes of bladder and can have an impact on the contractility of detrusor. *Neurourol. Urodynam. 19:29–42, 2000.*

Key words: bladder outlet obstruction; relief of obstruction; collagen types I and III; detrusor contraction

INTRODUCTION

Clinically, a bladder outlet obstruction results in alterations in structural and functional change in detrusor muscle. A partial obstruction of bladder outlet induces

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a rapid hypertrophy characterized by increased bladder mass and collagen deposition and decreased contractile property of detrusor muscle [Mayo et al., 1973; Uvelius et al., 1984; Uvelius and Mattiasson, 1984].

Collagen is the major constituent of the extracellular matrix in bladder. Bladder collagen has been suggested to influence the passive property of bladder wall [Kondo and Sussset, 1974], and collagen fibrils in smooth muscle probably also play an important role in intercellular transmission of active force [Gabella, 1977]. A previous report [Uvelius and Mattiasson, 1984] showed that although the total amount of collagen increased, the collagen concentration decreased in hypertrophied detrusor. However, this finding was not enough to explain that the increase in collagen amount affects the contractile property of smooth muscle.

The presence and characterization of different types of collagen in bladder were described [Ewalt et al., 1992]. Types I and III collagen appear to be the most abundant collagen types that significantly influence biological functions of the lower urinary tract. Types I and III collagen are fibrillar. Type I collagen provides the tensile strength of the tissue, while type III collagen is often associated with the striated fibrils of certain stromal matrix [Linsenmayer, 1991]. However, to date, the distribution and synthesis of different types of collagen after bladder outlet obstruction, especially after relief of obstruction, is not well understood. This study was undertaken to investigate the effects of partial bladder outlet obstruction and its relief on types I and III collagen, and the relationship between the detrusor muscle contractility and the changes in collagen types in partially obstructed rat bladder and in the bladder after the relief of obstruction.

MATERIALS AND METHODS

Animals and Tissue Preparation

A total number of 40 Sprague-Dawley female rats weighing 230–270 g were used for this experiment. The rats were divided into five groups: one control (n = 8) and four experimental groups (n = 32). For the control group, the bladders from un-operated rats were used. The experimental groups were one obstruction group (the rats with 6 weeks of partial bladder outlet obstruction) and three recovery groups (the rats 2, 4, and 6 weeks after the removal of ligature).

For each rat, after induction of general anesthesia with 15 mg/kg ketamine and 5 mg/kg xylazine intramuscularly, a suprapubic midline incision was made to expose the bladder and urethra. 3-0 silk ligature was placed around the catheterized urethra to create a partial outlet obstruction. Then, the catheter was removed and the incision was closed. Six weeks after the obstruction, the rat was again anesthetized, and the obstructing ligature was removed. The rats in the obstruction group were killed at this time; the rats in the recovery groups were killed at 2-week intervals after relief of obstruction at weeks 2, 4, and 6.

Each rat was killed by cervical fracture, and the bladder was separated between the base and body at the level of the ureteral orifices and weighed. The bladder body was cut longitudinally; one half was placed into Tyrode's solution (NaCl 124.9 m*M*, KCl 2.6 m*M*, $NaHCO_3$ 23.8 m*M*, $MgCl_2$ 0.5 m*M*, NaH_2PO4 0.4 m*M*, dextrose 5.5 m*M*, and $CaCl_2$ 1.8 m*M*) for muscle bath studies, and the other half was rapidly frozen in liquid nitrogen for analysis of collagen.

Isolated Muscle Strip Study

Two or three longitudinal strips (2 × 7 mm) were obtained from the one half of the bladder. Each muscle strip was placed in the bath containing 30 mL Tyrode's solution aerated with a mixture of 95% O_2 + 5% CO_2 and maintained at 37°C. Preliminary length-tension studies demonstrated that 1 *g* of passive tension was required to generate maximum active tension. Each strip was subjected to this tension, and equilibrated for 30 minutes before study of its contractility. The maximal response of each strip to field stimulation (80 V, 2–32 Hz, 1 ms) was determined. Contractile responses were monitored via a FT 0.03 force transducer and recorded on a Grass 7D polygraph and expressed as grams tension per 100 mg.

Collagen Determination

A bladder specimen, weighing approximately 25 mg, was used to determine the tissue collagen concentration; the concentration was determined by estimating hydroxyproline content (percent collagen = 7.46 × percent hydroxyproline) [Neuman and Logan, 1950]. The collagen was extracted in distilled water and hydrolysed in 6 *M* HCl as described by Grant [1964]. The hydroxyproline content was then determined by the modified Stegemann procedure [Bergman and Loxley, 1963], using a spectrophotometer.

Immunohistochemical Staining

Rabbit anti-rat polyclonal antibodies to types I and III collagen (Biodesign, Kennebunk, ME) were used as the primary antibodies in this study. Rabbit anti rat collagen type III has been reported to have <10% cross-reactivity with rat collagen type V. Positive controls were sections of rat skin and negative controls were obtained by omitting primary antibodies. The 3-μm sections of the fresh frozen bladder specimen were mounted on glass slides, air dried, and pre-treated with acetone (−20°C) for 10 minutes. The sections were incubated with antibodies types I and III, diluted 1:100 and 1:400, respectively, with 0.15 *M* NaCl in 0.05 *M* Tris-HCl buffer (pH 7.5) in a humid chamber for 40 minutes at room temperature and were then rinsed in several changes of pH 7.6 cold phosphate-buffered saline (PBS). The sections were incubated with goat anti-rabbit immunoglobulin G (Vector Lab., Inc., Burlingame, CA) for 25 minutes in the same diluent used for the primary antibodies. After the slides were washed with cold PBS, they were counterstained using standard hematoxylin and eosin for 3 minutes and were coverslipped.

RNA Isolation

Total RNA was extracted from the rat urinary bladder tissues using a RNeasy mini kit (Qiagen, Inc., Hilden, Germany). The rapid isolation of total RNA procedure was used to minimize loss of RNA during purification. RNA samples were tested with ultraviolet absorption A260/A280 for purity and concentration. Values for the A260-to-A280 ratios were >1.8 for all RNA extraction. The quality and concentration of the RNA samples were further confirmed by electrophoresis on denatured 1% agarose gels [Lehrach et al., 1977].

Northern Blot Analysis

The quantity of mRNA expression in the tissue was measured using the Northern blot analysis. Total RNA (4 μg) from each sample was electrophoresed on 1% denatured agarose gel and transferred onto a nitrocellulose membrane. The pro-α1(I) and α1(III) collagen complementary DNA fragments amplified with the polymerase chain reaction were used to measure the pro-α1(I) and α1(III) collagen mRNA, respectively. Complementary DNA fragments were labeled with digoxigenin-11-2′deoxyuridine-5′triphosphate (DIG) (Boehringer-Mannheim, Mannheim, Germany), and the Northern blots were probed overnight. The blots were then washed and incubated with the alkaline phosphatase-conjugated anti-DIG Fab fragment solution for 30 minutes. The blots were incubated with CSPD, chemiluminescent substrate for 15 minutes and exposed to x-ray film for 10 minutes to desired signal levels. Signal intensity was analyzed using a densitometer (ImageMaster VDS, Pharmacia Biotech Inc., San Francisco, CA) and normalized against beta-actin mRNA.

Statistical Analysis

Data are expressed as means ± SEM. Comparisons between groups were made using the analysis of variance (ANOVA) followed by the Newman-Keul's test for multiple comparisons. A P value <0.05 was considered significant.

RESULTS

Bladder Mass

The bladder mass increased significantly after 6 weeks of partial outlet obstruction but decreased after relief of obstruction. However, it did not return to the pre-obstruction level at the 6 weeks recovery period (Fig. 1).

Isolated Muscle Strip Study

The contractile responses to field stimulation are shown in Fig. 2. The contractile response was significantly decreased after 6 weeks of partial outlet obstruction. After the 2-week recovery period, it did not change significantly compared with the obstruction group. However, it changed significantly at the 4- and 6-week recovery period compared with the obstruction group, although the contractile response at the 6-week recovery period did not return to the pre-obstruction level (Fig. 2).

Analysis of Collagen

The results from the collagen determinations are presented in Table I. The total amount of collagen in the detrusor muscle increased significantly 6 weeks after partial outlet obstruction but decreased after the relief of obstruction. The decrease was significant 6 weeks after relief. However, the collagen concentration decreased significantly 6 weeks after partial outlet obstruction and increased after its relief. The increase of collagen concentration was significant 6 weeks after relief.

The regression analysis between contractile response to 32-Hz field stimulation and collagen content is shown in Fig. 3. The contractile response decreased with

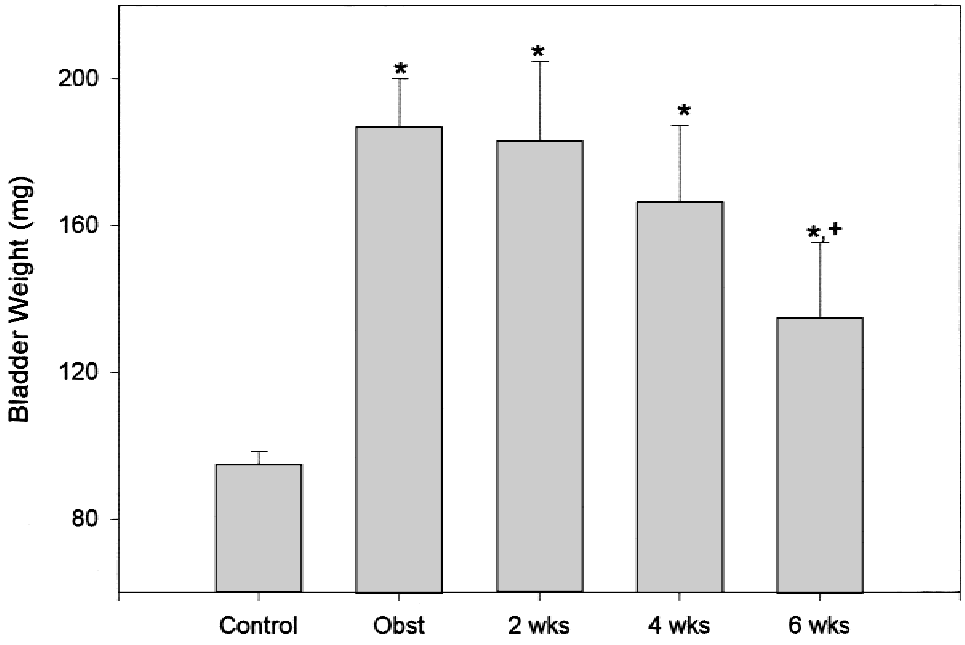

Fig. 1. Changes of bladder weight in control, obstruction (obst) and 2, 4, and 6 weeks (6 weeks) after the relief of obstruction. $*P < 0.05$, compared with control value. $+P < 0.05$, compared with obstruction value.

increasing total amount of collagen (Fig. 3A) but increased with increasing collagen concentration (Fig. 3B).

Immunohistochemical Staining

In a normal bladder, the protein deposition for types I and III collagen was mostly localized in the lamina propria. With obstruction they were more localized between smooth muscle bundles. Furthermore, type III collagen was partially localized within muscle bundles in some specimens. After the relief of obstruction, the protein deposition tended to return to normal control, especially in muscle layers (Fig. 4A,B).

Analysis of Types I and III Collagen mRNA

There was up regulation of the pro-α1(I) and α1(III) gene expression after the obstruction with more profound pro-α1(III) gene expression. After the relief of obstruction, the increased gene expressions were down regulated and more prevalent in the pro-α1(I) collagen gene expression. Pro-α1(I) gene expression decreased to normal control value but pro-α1(III) gene expression did not return to control value at the 6-week recovery period (Figs. 5 and 6). The regression analysis between the contractile response to 32-Hz field stimulation and pro-α1(I) and α1(III) gene expression is shown in Fig. 7. The contractile response decreased with increasing pro-α1(I) gene expression (Fig. 7A) and decreased with increasing pro-α1(III) gene expression (Fig. 7B).

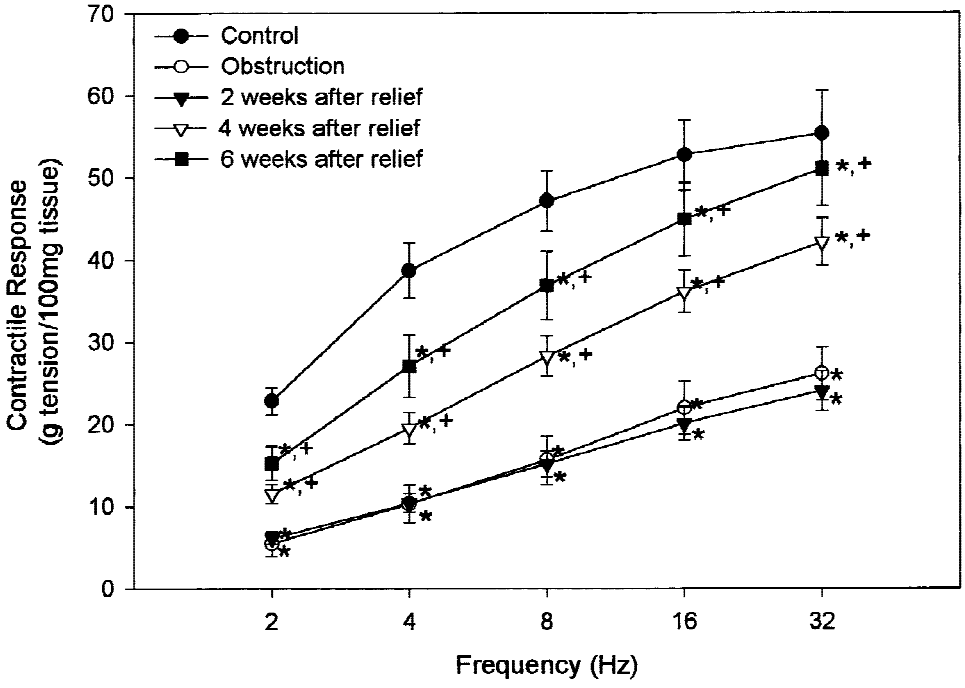

Fig. 2. Contractile response of bladder muscle strip to 2-, 4-, 8-, 16-, and 32-Hz field stimulation in control, obstruction and 2, 4, and 6 weeks after relief of obstruction. *$P < 0.05$, compared with control value. +$P < 0.05$, compared with obstruction value.

DISCUSSION

Bladder outlet obstruction by prostate hypertrophy produces morphological, biochemical, and functional changes [Malkowicz et al., 1986; Kitada et al., 1989; Kato et al., 1990b; Levin et al., 1992]; these changes result in the impairment of detrusor contractility. In most patients with this bladder outlet obstruction, the removal of the outflow obstruction restores the bladder function. However, in approximately 23% of the patients the unstable bladder contraction persists [Turner-Warwick, 1984].

The increase in the bladder mass is one of the most noticeable responses of the

TABLE I. Total Collagen and Collagen Concentration in Control, Obstruction and 2, 4 and 6 Weeks After the Relief of Obstruction

Group	Total collagen (mg)	Collagen concentration (μg/mg)
Control (n = 8)	5.2 ± 0.2	44.0 ± 1.6
Obstruction (n = 8)	7.3 ± 0.4*	34.6 ± 1.5*
2 weeks after relief (n = 8)	6.6 ± 0.7	37.6 ± 2.0
4 weeks after relief (n = 8)	6.2 ± 0.5	37.2 ± 1.3
6 weeks after relief (n = 8)	5.4 ± 0.5**	40.9 ± 1.2**

*$P < 0.05$, compared with control value.
**$P < 0.05$, compared with obstruction value.

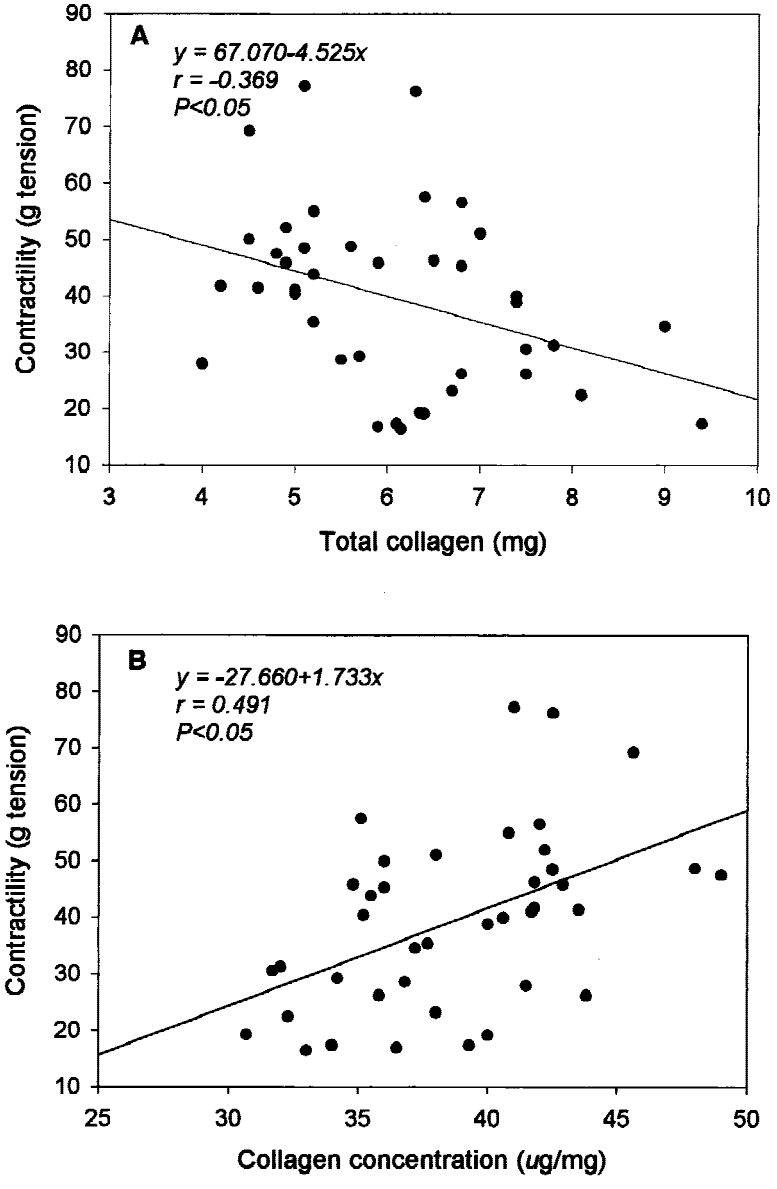

Fig. 3. Regression analysis for the effect of collagen content on the bladder muscle contractility. **A:** The effect of total collagen amount on the contractility to 32-Hz field stimulation. **B:** The effect of collagen concentration on the contractility to 32-Hz field stimulation.

bladder to an experimentally induced outlet obstruction. Lindner et al. [1988] reported that the weight of detrusor increased 10-fold after 6 weeks of obstruction and decreased rapidly after the removal; however, the weight did not come down to the pre-ligature weight. In our series, the weight of detrusor increased twofold 6 weeks after the obstruction compared to 10-fold reported by Lindner et al. [1988]; this difference may be due to the ligatures being lightly tied. Also, the weight of detrusor did not return to the pre-ligature weight as reported by Lindner et al. [1988].

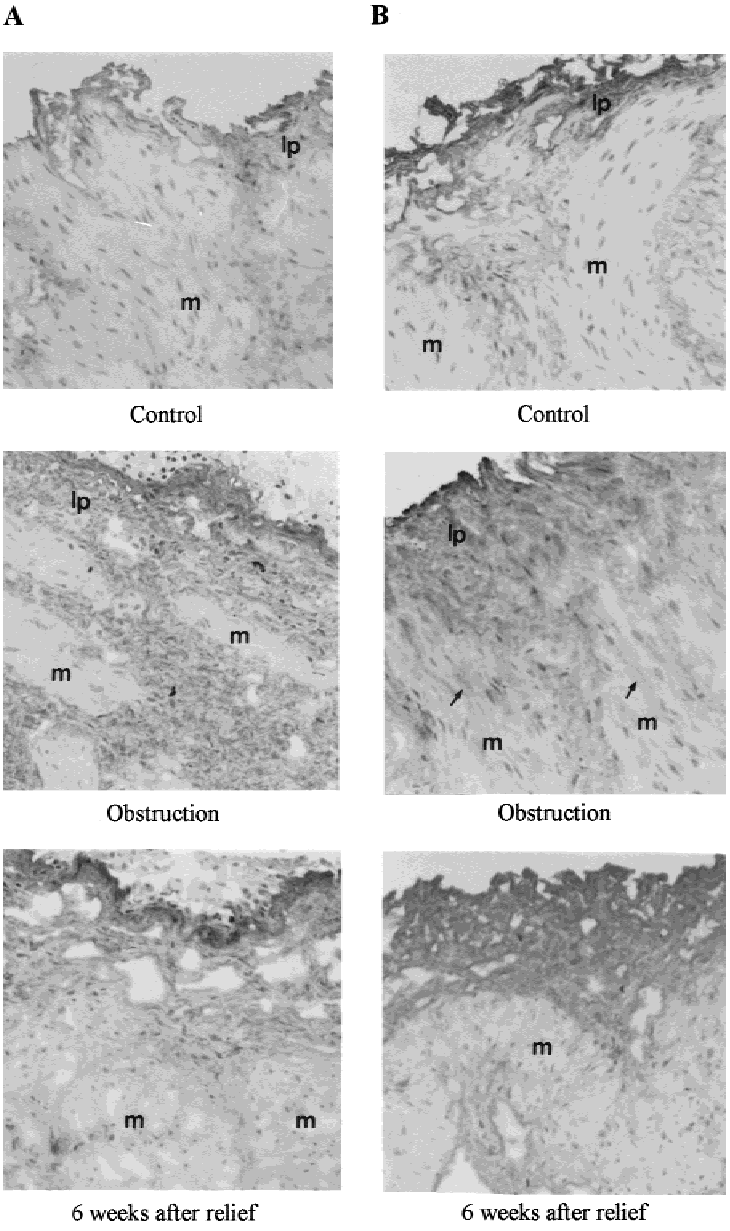

Fig. 4. Immunohistochemical staining with types I **(A)** and III collagen **(B)** in control, obstruction, and 6 weeks after relief groups. The protein deposition for types I and III collagen was mostly localized in the lamina propria (lp). With obstruction they were more localized between smooth muscle bundles (m). Furthermore, type III collagen was partially localized within muscle bundles (arrow). After relief, the protein deposition tended to return to normal control, especially in muscle layers. Original magnification ×200.

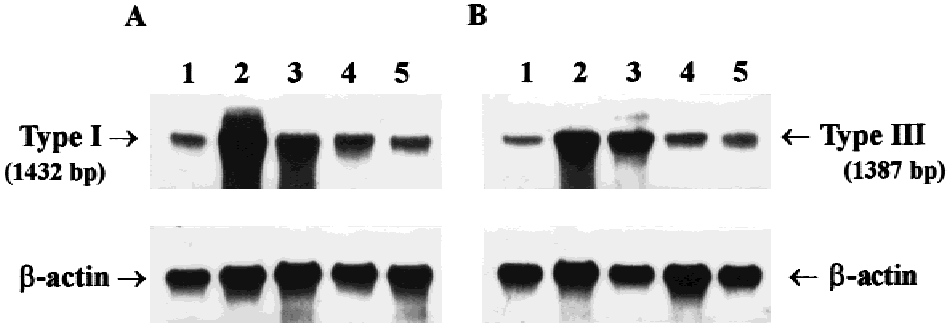

Fig. 5. Representative autoradiographs from northern blot hybridization of total RNA for pro-α1(I) collagen mRNA **(A)** and pro-α1(III) collagen mRNA **(B)**. Lane 1, control; lane 2, obstruction; lane 3, 2 weeks after relief; lane 4, 4 weeks after relief; lane 5, 6 weeks after relief.

When an outlet obstruction is produced in experimental animals, the ability of bladder tissue to respond to field stimulation is decreased markedly [Malkowicz et al., 1986; Kato et al., 1990a; Levin et al., 1990]. Levin et al. [1985] reported contractile defects reverse after removal of obstruction. However, in our series, the contractile response to field stimulation did not improve to the control level by 6 weeks of

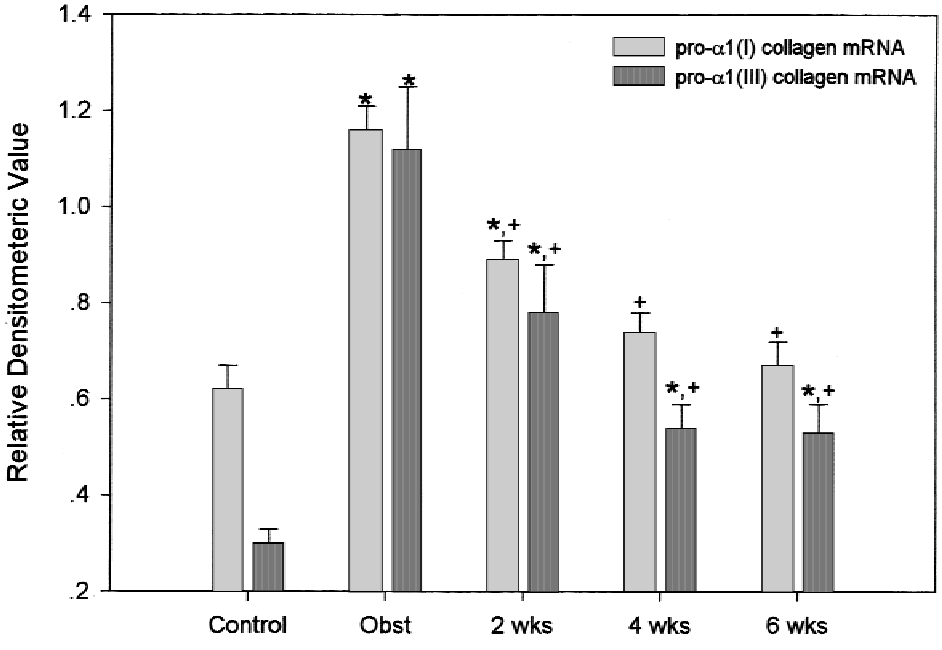

Fig. 6. Results of densitometric scanning of autoradiograms from northern blot hybridizations of total RNA from bladder tissue of rats in control, obstruction (obst) and 2, 4, and 6 weeks (6 weeks) after the relief of obstruction. The density of an individual mRNA band was divided by the density of the corresponding β−actin mRNA band. All data are presented as a relative densitometric value to the corresponding β−actin mRNA. $^{*}P<0.05$, compared with control value. $^{+}P<0.05$, compared with obstruction value.

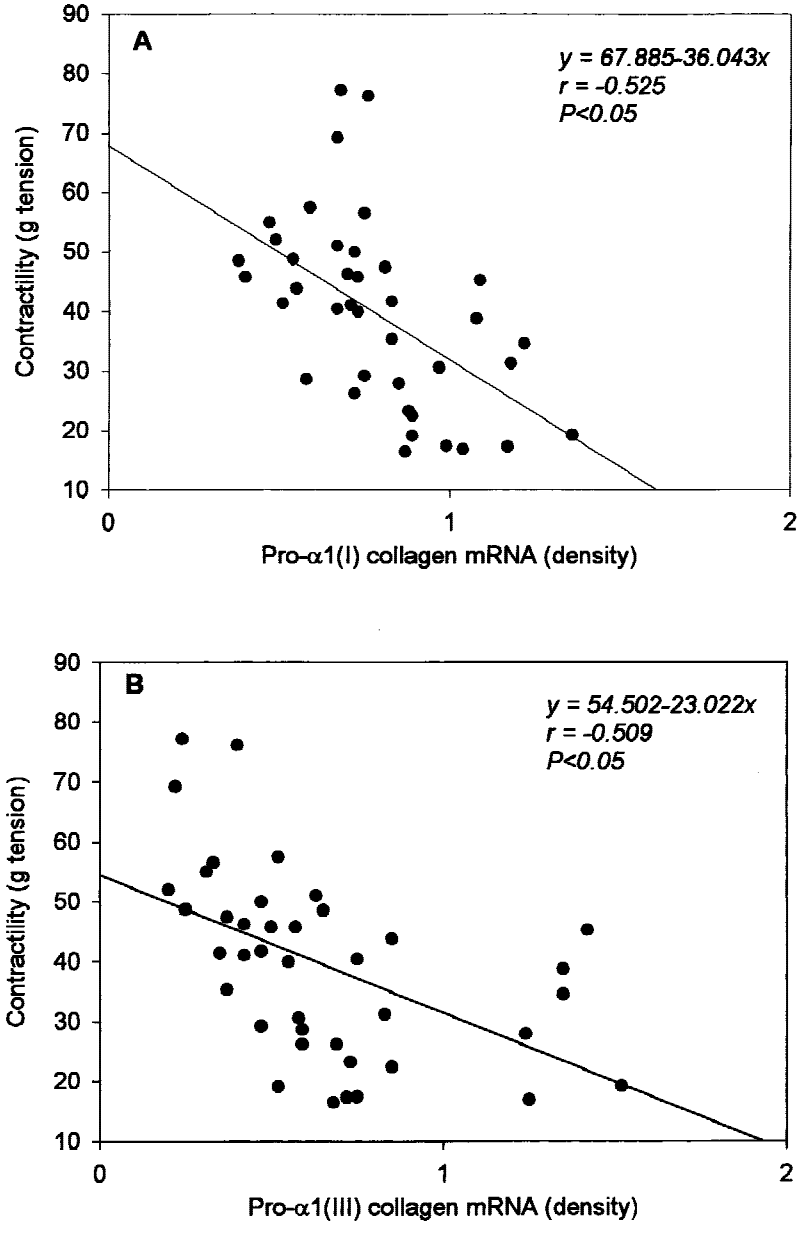

Fig. 7. Regression analysis for the effect of collagen mRNA on the bladder muscle contractility. **A:** The effect of pro-α1(I) collagen mRNA on the contractility to 32 Hz field stimulation. **B:** The effect of pro-α1(III) collagen mRNA on the contractility to 32 Hz field stimulation.

recovery. This result may indicate that the obstruction caused a prolonged minor defect in the ability of bladder to contract.

Bladder outlet obstruction induces changes within the extracellular matrix; and the essential part of these changes appears to be collagen deposition in smooth muscle. Collagen fibrils in smooth muscle probably also play an important role in

intercellular transmission of active force [Gabella, 1977]. Thus, a change in collagen content might also affect the contractile property of the smooth muscle. Uvelius and Mattiasson [1984] reported that in the obstructed rat bladder, collagen content increased, while the concentration of the collagen decreased. The decrease in the collagen concentration in an obstructed rat bladder has been explained by the relative decrease in interstitial tissue rich in collagen and by an increase in the amount of smooth muscle bundles where collagen seemed less abundant. After obstructing ligature removal, collagen content decreased, while its concentration increased [Uvelius et al., 1991]. The results of the present study agree with those of others [Uvelius and Mattiasson, 1984; Uvelius et al., 1991]. The present study investigated the associations among the detrusor contractility, the collagen content and collagen concentration. It showed that the detrusor contractility correlated negatively with collagen content but correlated positively with collagen concentration. This result is not enough to explain that the increase in collagen deposition following outlet obstruction affects the contractile property of the smooth muscle. Thus, the fact that the changes of the collagen types affect the functional ability of smooth muscle was expected.

Types I and III collagen appear to be the most abundant collagen types that significantly influence the biological function of the lower urinary tract. An abnormality in the production or distribution of different types of collagen has a profound impact on mechanical properties of bladder connective tissues [Shapiro et al., 1991; Ewalt et al., 1992; Baskin et al., 1994; Landau et al., 1994]. Tekgul et al. [1996] reported an up regulation of types I and III collagen gene expression after obstruction in muscular layer, and protein deposition for both collagen types was analogous to that of gene expression. The staining for type I collagen increased in intensity between smooth muscle bundles; the staining for type III collagen increased in intensity in and around muscle bundles. The present study showed similar results with those from Tekgul et al. [1996]. However, after the relief of obstruction, the present study showed the localization of types I and III collagen tended to return to normal control, especially in muscular layers. It might be assumed from our study that the localization of collagen types could be normalized if the recovery period had been longer. Moreover, this finding indicates that the change of the collagen types in muscular layer may affect the contractility of the bladder after obstruction and its relief.

There has been little study of quantitative changes of collagen types after partial outlet obstruction, especially after the relief of obstruction. To our knowledge, the present study is first study to observe the quantitative change of gene expression for collagen types after relief of obstruction and the correlation between mRNA expression and detrusor contractility. The expression of pro-collagen mRNA may not reflect the deposition of collagen completely because the deposition of collagen is affected by various factors as well as transcription of collagen gene. However, it was suggested that increased transcription of collagen genes may be responsible for the accumulation of these collagen and biosynthesis of collagen is proportional to the amount of pro-collagen mRNA [Bashey et al., 1993; Uitto, 1993]. Accordingly, It is possible to infer the content of collagen types by measuring the degree of collagen gene expression.

Densitometric scans of pro-α1(I) and α1(III) collagen mRNA was normalized to the β-actin mRNA. Although the relevance for the multiplicity of isoactins in vertebrates is not clear, it has been reported that the relative amount of each isoactin in a cell is influenced by cellular changes [Schevzov et al., 1992]. In general, the dominant

type of actin in the smooth muscle is γ-actin, and β-actin exists in small amount as cytoplasmic type [Gabbiani et al., 1984]. After bladder outlet obstruction, the major portion of changes is γ-actin. Although there is some controversy whether the actin isoforms are increased or decreased after outlet obstruction, it may be true that some change of β-actin appears [Malmqvist et al., 1991; Kim et al., 1994]. In our study, the changes of β-actin might be very little because of the small degree of muscle hypertrophy. Therefore, we think that it makes no difference for normalizing to β-actin mRNA. However, it would be better if 18S rRNA or GAPDH whose expression would not be expected to change in response to obstruction was used. The gene expression of pro-α1(I) and α1(III) increased after obstruction, especially more rapidly in pro-α1(III). After the relief of obstruction, the increased gene expression decreased rapidly; especially, pro-α1(I) expression was normalized to the control level. However, Pro-α1(III) expression did not significantly normalized to the control level. This finding indicates that the gene expression of collagen decreases more rapidly in the transcriptional phase than the decrease of muscle mass in the recovery period of detrusor function. Also, it is assumed that no recovery of pro-α1(III) gene expression may cause incomplete recovery of contractility of detrusor as shown in isolated muscle strip study. We investigated the relationship between the detrusor contractility and the gene expressions of collagen types. The result showed that the detrusor contractility correlated negatively with pro-α1(I) and α1(III) expression. The present study, therefore, demonstrated that the change in the collagen gene expressions influences the detrusor contractility in partially obstructed rat bladder and in the bladder after the relief of obstruction. The introduction of transforming growth factor-β and epidermal growth factor was shown to influence the different types of collagen [Federspiel et al., 1991; Howlett and Bissell, 1993; Alison and Sarraf, 1994]. Further investigations are needed to determine whether the collagen expression in bladder is also regulated by any growth factor.

CONCLUSIONS

In conclusion, partial bladder outlet obstruction and its relief affect on changes of localization and quantity of collagen types as well as collagen content, and these changes lead to bladder remodeling. Moreover, our results suggest that the change in the collagen types rather than the change in the collagen content has an impact on the detrusor contractility.

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