Persistent detrusor overactivity in rats after relief of partial urethral obstruction

Long-Hu Jin,1 Karl-Erik Andersson,4 Jeong-Uk Han,2 Yong-Hyun Kwon,1 Chang-Shin Park,3 Hwa-Youan Shin,1 Sang-Min Yoon,1 and Tack Lee4

Departments of 1Urology, 2Anesthesiology, and 3Pharmacology, Inha University College of Medicine, Incheon, Korea; and 4Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Winston Salem, North Carolina

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Jin LH, Andersson KE, Han JU, Kwon YH, Park CS, Shin HY, Yoon SM, Lee T. Persistent detrusor overactivity in rats after relief of partial urethral obstruction. Am J Physiol Regul Integr Comp Physiol 301: R896–R904, 2011. First published July 27, 2011; doi:10.1152/ajpregu.00046.2011.—Detrusor overactivity (DO) persists after prostatectomy in 20% to 25% of patients with benign disease. Assuming that nonvoiding contractions (NVCs) can be used as a surrogate for DO in humans, the rat model of obstruction/deobstruction may allow us to study the pathophysiology of persistent DO after deobstruction. We investigated bladder function, with a special focus on NVCs, in rats by use of a new, modified method of obstruction and deobstruction and compared these results with those obtained by use of the conventional method. Seventy female Sprague-Dawley rats underwent sham operation (n = 10), 2 obstruction by a modified method (Modif-Obs; n = 12), 3 obstruction/deobstruction by the conventional method (Conv-Obs/Deobs; n = 13), or 4 obstruction/deobstruction by the modified method (Modif-Obs/Deobs; n = 35). The Modif-Obs/Deobs animals were divided into subgroups with (DO+) and without (DO−) NVCs. Two weeks after partial urethral obstruction, the animals were deobstructed, and 1 wk later cystometry was performed with recording of intravesical and intra-abdominal pressures. NVCs were shown in all groups: Modif-Obs (80%), Conv-Obs/Deobs (100%), and Modif-Obs/Deobs (40%). In the Modif-Obs/Deobs group, bladder weight and the muscle-to-collagen ratio were higher in DO+ than in DO− rats. The Modif-Obs/Deobs group showed no mortality compared with 25% mortality in the Conv-Obs/Deobs group. The modified method may be more adequate for studying persistent DO after deobstruction, because it resulted in pressure/volume- and DO-related parameters similar to those found in the clinical situation. The persistence of DO after deobstruction may partly be due to irreversible changes in the bladder caused during the period of obstruction.

urinary bladder; cystometry

BENIGN PROSTATIC HYPERPLASTIA occurs as part of the aging process in men and affects >50% of the male population over 60 yr of age (11). The clinical symptoms of benign prostatic hyperplasia are caused not only by the mass-related increase in urethral resistance but also by pathologic processes in the bladder wall secondary to the bladder outlet obstruction (BOO) (18, 26, 35, 40). In many studies on patients with BOO undergoing surgery for benign prostatic hyperplasia, a significant percentage of patients report persistence of storage (irritative) symptoms, associated with detrusor overactivity (DO) on urodynamical investigation, even if the obstruction was successfully relieved. In fact, DO has been shown to persist after prostatectomy in 20% to 25% of patients treated for benign disease (2, 19, 29, 33).

Rats subjected to BOO will develop hyperactive voiding, characterized by increased frequency and nonvoiding contractions (NVCs), which persists after deobstruction in a proportion of the animals (6, 20, 23, 24, 27, 30). The relations between symptoms, BOO, and bladder function can, for obvious reasons, not be studied in animal models. However, assuming that NVCs can be used as a surrogate parameter for DO in humans, the rat model of obstruction/deobstruction may offer an opportunity to study the pathophysiology of persistent DO after deobstruction. Many studies have been performed on the morphological and functional bladder changes that occur after deobstruction (6, 10, 21, 25, 34), but to the best of our knowledge, no studies have focused on NVCs and factors that can influence this outcome parameter, which may have translational value.

To study bladder function in conscious, deobstructed rats by use of conventional obstructing methods, three abdominal openings are needed 1) at the time of obstruction, 2) at the time of deobstruction, and 3) at the time of bladder catheterization (6, 25). The repeated incisions make it difficult to compare voiding in the obstructed and deobstructed rats and may increase the complication rate. In fact, deobstructed rats showed a 25% mortality rate in a study by Chai et al. (6). Therefore, we designed a model of BOO in female rats in which the knot of an obstructing silk suture is placed in the vaginal lumen. The ligature is then easily removed through a vaginal approach without the need to open the abdomen at the time of deligation.

In most animal models, true bladder (detrusor) pressure is not recorded, because this requires intra-abdominal pressure (IAP) measurement. Lack of IAP recording could be a source of error when studying DO, because conscious animals are physically active with consequent increases in IAP/intravesical pressure (IVP), and this may occur without concomitant changes in detrusor activity. In the present study, we therefore measured IAP and IVP simultaneously by use of a previously described technique (18).

Many studies on the morphological changes in the bladder in the presence of BOO have been performed in experimental animal models and in humans (15, 32, 39). The accumulating evidence obtained from these studies suggests that BOO is followed by an increased thickness of the bladder wall with hypertrophic changes to the detrusor smooth muscle (9, 12, 39) and an altered amount of collagen tissue (8, 37, 38). Although the role of connective tissue in bladder function has not been clarified in detail, many previous results suggest that an appro-
appropriate balance between these two components of the bladder wall may be of great relevance in achieving normal bladder function (14, 26, 35).

The aim of this study was to further study bladder function, with special focus on NVCs, in obstructed/deobstructed rats. To reduce the influence of confounding factors, we used a modified method of obstruction/deobstruction and simultaneous recording of IVP and IAP. These results were compared with those obtained by use of the conventional method. Furthermore, we attempted to reveal possible changes in the amount of connective tissue relative to detrusor smooth muscle by morphometric analysis in rats with obstruction and obstruction/deligation.

MATERIALS AND METHODS

Experimental animals. Adult female Sprague-Dawley rats obtained from Orient Bio (GyeongGi, Korea) and weighing 210 to 250 g were housed in a vivarium with free access to water and food with diurnal light cycling. After surgery, the animals were caged individually and maintained in the same manner. All procedures for animal handling and treatment were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and were approved by the Ethics Committee of the Inha University College of Medicine.

Seventy rats were randomly separated into four groups. The first group consisted of control, sham-operated rats (n = 10). The second group underwent a modified method of urethral constriction to produce partial bladder obstruction (Modif-Obs), which will be described in the following section (n = 12). The third group underwent the modified method of urethral constriction, and after 2 wk, deobstruction through the vaginal route without an additional abdominal incision (Modif-Obs/Deobs; n = 35). The fourth group underwent urethral constriction by the conventional method (4, 24, 28) and then deobstruction through an additional abdominal incision after 2 wk (Conv-Obs/Deobs; n = 13). On the basis of the cystometric criteria for the presence of DO during the filling phase, the Modif-Obs/Deobs group was divided into subgroups showing DO (DO+) or not showing DO (DO−). DO in this study was defined as the occurrence of NVCs.

Conventional surgical method. Anesthesia was induced by ketamine (75 mg/kg ip Ketamine 50; Yuhan, Korea) and xylazine (15 mg/kg ip Rompun; Bayer Korea). Through a lower midline incision, the bladder was approached, and the proximal urethra exposed. A 3-0 Novafil (monofilament polybutester; Davis & Heck, Wayne, NJ) ligature was placed around the urethra and tied in the presence of an intraluminally placed steel rod with a diameter of 0.9 mm. After the knot was tied, the steel rod was removed, the bladder was repositioned, and the abdominal wall was closed.

Modified surgical method. Through a lower abdominal midline incision, the retropubic space was exposed and a 4-0 silk stay suture was placed about 3 mm lateral to the urethra to pull up the vaginal epithelium to make it easy to incise. After a small incision was made, the needle of a 3-0 nylon ligature was inserted through the incision, passed via the vaginal space, and taken out laterally on the other side. The nylon ligature was tied gently around the urethra with the vaginal epithelium in the presence of a 1-mm steel rod placed alongside the urethra. After the knot was tied, the steel rod was removed and the two ends of nylon from the knot were pulled down into the incision, and the knot was rolled down and positioned in the vaginal space, which made it easy to be removed through the vaginal approach (Fig. 1).

Cystometric investigations were performed without anesthesia 2 wk after the first surgery in the sham and Modif-Obs groups and 1 wk after the deobstruction in the Modif-Obs/Deobs and Conv-Obs/Deobs groups. The catheters were implanted 3 days before the cystometrics.
was assumed to be 100% in each specimen. The areas of muscle and collagen were expressed as a percentage of total area.

Statistical analysis. The results are given as means \( \pm \) SE. Normal distributions were confirmed by the Shapiro-Wilks’ W test. Statistical analyses were undertaken with unpaired Student’s t-tests or a one-way ANOVA with the Tukey post hoc test for multiple comparisons. The percentage data for muscle and collagen areas were checked for normality using the Kolmogorov-Smirnov test (\( P < 0.05 \)). The analysis showed that the difference between the distribution of the analyzed data and the normal distribution was not statistically significant (\( P > 0.05 \)). Statistically significant differences were calculated by one-way ANOVA followed by Tukey’s test for the comparison of percentage data. All analyses were performed with GraphPad Prism, version 5.03, 2009 (Graph Pad Software, San Diego, CA). \( P < 0.05 \) was considered statistically significant.

RESULTS

During the course of the experiment, two (17%) rats in the Modif-Obs group died, five (14%) in the Modif-Obs/Deobs group died, and four (31%) in the Conv-Obs/Deobs group died. However, the Modif-Obs/Deobs group showed no mortality after deobstruction among the 30 rats that survived the obstruction. The Conv-Obs/Deobs group showed a mortality rate of 25% after deobstruction among the 12 rats that survived the obstruction (Table 1).

Representative cystometric tracings from rats in the sham, Modif-Obs, Conv-Obs/Deobs, and Modif-Obs/Deobs groups are shown in Fig. 3. Both the filling and the voiding phases of micturition could be reproducibly recorded in the animals. In the Modif-Obs/Deobs group (\( n = 30 \)), we subclassified the animals as DO positive (DO\(^+\); \( n = 12 \)) or DO negative (DO\(^-\); \( n = 18 \)) after deobstruction as judged by simultaneous changes in IAP and IVP.

Body and bladder weights. There were no significant differences in body weight among any of the groups on the day of cystometry (data not shown). All of the groups with obstruction showed an increase in the ratio of bladder (mg) to body (g) weight compared with that in the sham group (\( P < 0.001 \)). In the Modif-Obs/Deobs group, the DO\(^+\) animals showed a higher ratio than did the DO\(^-\) subgroup (\( P < 0.01 \)) (Fig. 4).

Morphology. Microscopically, smooth muscle bundles were separated from each other by loose collagen fibers (Fig. 5A). In the sham group, the average percentage of smooth muscle and collagen over the sum of both was 33.1 \( \pm \) 5.0% and 66.9 \( \pm \) 5.0%, respectively. After obstruction, the percentage of smooth muscle (63.4 \( \pm \) 2.7%) increased (\( P < 0.05 \)), but that of collagen decreased (36.6 \( \pm \) 2.7%, \( P < 0.05 \)) compared with the sham group. After deligation, the DO\(^+\) group showed a higher percentage of smooth muscle (71.5 \( \pm \) 1.2%, \( P < 0.001 \)) and a lower percentage of collagen (28.5 \( \pm \) 1.2%, \( P < 0.001 \)) compared with the values during obstruction. The DO\(^-\) group, however, showed no significant differ-
Compared with that in sham animals, BP was elevated in the B groups (Fig. 5 compared with the values of the sham and Modif-Obs groups). The Modif-Obs group did not show any significant difference in MP compared with the sham group, but MP was higher in the Conv-Obs/Deobs (P < 0.01) and Modif-Obs/Deobs groups (P < 0.05) and in the DO+ subgroup (P < 0.01) than in sham animals. MP was lower in the DO− subgroup than in the Conv-Obs/Deobs (P < 0.01) and DO+ animals (P < 0.01) (Fig. 6B).

Compliance was increased in all obstructed groups compared with the sham group. Compared with the Modif-Obs animals, compliance was lower in the Conv-Obs/Deobs animals (P < 0.001) and higher in the Modif-Obs/Deobs (P < 0.001) group. Compliance was lower in the Conv-Obs/Deobs group than in Modif-Obs/Deobs (P < 0.001) rats. There was no significant difference in compliance between the DO+ and DO− subgroups (Fig. 6D).

BC was higher in the Modif-Obs (P < 0.001) and Modif-Obs/Deobs (P < 0.01) groups than in sham animals. This was due to increased RV (P < 0.01) in the Modif-Obs group and was associated with an increased MV (P < 0.001) in the Modif-Obs/Deobs group. However, compared with the sham rats, the Conv-Obs/Deobs group showed no increase in BC (P > 0.05). There was a significant difference in RV between the DO+ and DO− subgroups (P < 0.05), although BC did not differ significantly between these subgroups (Fig. 7). MI was higher in the Modif-Obs (P < 0.001) and Modif-Obs/Deobs (P < 0.01) groups, but not in the Conv-Obs/Deobs group, than in the sham group. There was no significant difference in MI between the DO+ and DO− subgroups (data not shown).

**Parameters to investigate DO during the filling phase.** Whereas the sham group showed no NVCs during the filling phase, the Modif-Obs, Conv-Obs/Deobs, and Modif-Obs/Deobs groups did. NVCs were demonstrated in eight of ten rats in the Modif-Obs group (80%), in all animals in the Conv-Obs/Deobs group (100%), and in 12 of 30 rats in the Modif-Obs/Deobs (DO+ subgroup; 40%) group. There

![Fig. 2. Representative cystometric recordings showing nonvoiding contractions (NVCs) and abdominal straining reflected by changes in intravesical pressure (IVP) and intra-abdominal pressure (IAP). A: an NVC was defined as increments of IVP that exceeded 2 cmH2O from baseline without simultaneous changes in IAP and without fluid expulsion from the bladder. DP, detrusor pressure. †NVC; ‡NVC pressure from the baseline to the peak of the NVC. B: note the abdominal straining, defined as elevations in IVP with simultaneous changes in IAP.](http://ajpregu.physiology.org/)

**Table 1. Mortality of the investigated rats according to the subgroup and procedures**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Modif-Obs</th>
<th>Modif-Obs/Deobs</th>
<th>Conv-Obs/Deobs</th>
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</thead>
<tbody>
<tr>
<td>Total number, investigated</td>
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<td>12</td>
<td>35</td>
<td>13</td>
</tr>
<tr>
<td>Mortality, total</td>
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<td>2 (16.7%)</td>
<td>5 (14.3%)</td>
<td>4 (30.8%)</td>
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<tr>
<td>Mortality, after obstruction</td>
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<td>2 (16.7%)</td>
<td>5 (14.3%)</td>
<td>1 (7.7%)</td>
</tr>
<tr>
<td>Mortality, after deobstruction</td>
<td>0</td>
<td>0 (0%)</td>
<td>3 (25%)</td>
<td></td>
</tr>
<tr>
<td>Real number, investigated</td>
<td>10</td>
<td>10</td>
<td>30</td>
<td>9</td>
</tr>
</tbody>
</table>

Numbers in parentheses are mortality rate. Modif-Obs, rats with partial outlet obstruction by the modified method; Modif-Obs/Deobs, rats obstructed and then deobstructed by use of our modified method; Conv-Obs/Deobs, rats obstructed and then deobstructed by use of the conventional method.
were no NVCs in the DO− subgroup. The Modif-Obs/Deobs group showed a lower frequency of NVCs than did the Conv-Obs/Deobs group but did not differ significantly from the Modif-Obs group. DO pressure was lower in the Modif-Obs/Deobs group than in the Modif-Obs group but did not differ significantly from that in the Conv-Obs/Deobs group (Fig. 8).

DISCUSSION

Even after surgical relief of BOO, 20% to 25% of men experience persistent DO and storage symptoms (2, 29, 33). The reasons for this have not been established. In animal models designed to investigate directly the postobstruction state of the bladder by use of cystometry, the conventional methods of obstruction and deobstruction have a potential for surgical morbidity and mortality owing to the accumulated operation hazards (6). This increased morbidity and mortality may have consequences for the urodynamic results. Another issue is the question of what, from a translational point of view, is relevant to measure in the animal model. DO is defined by the International Continence Society as a urodynamic observation characterized by involuntary detrusor contractions during the filling phase that may be spontaneous or provoked (1). In cystometric animal models it is, for obvious reasons, not possible to establish whether a detrusor contraction is involuntary or not. However, in animals, the presence of detrusor contractions during the filling phase (NVCs) may be a reason-

Fig. 3. Representative cystometric tracings in conscious rats from various groups. A: sham-operated rats. Vol, volume. B: rats with partial outlet obstruction by means of the modified method (Modif-Obs). C: rats obstructed and then deobstructed by means of the conventional method. D: rats obstructed and then deobstructed by means of our modified method (Modif-Obs/Deobs) showing NVCs during filling. E: Modif-Obs/Deobs rats showing no DO.

Fig. 4. Effect of partial outlet obstruction, deobstruction, and NVCs on the ratio of bladder (mg) to body weight (g). Vertical bars represent mean results ± SE. **P < 0.01; ***P < 0.001. Modif-Obs, rats with partial outlet obstruction by means of the modified method; Conv-Obs/Deobs, rats obstructed and then deobstructed by means of the conventional method; Modif-Obs/Deobs, rats obstructed and then deobstructed by means of the modified method; DO+, Modif-Obs/Deobs rats showing NVCs; DO−, Modif-Obs/Deobs rats showing no NVCs; NS, not significant.
able surrogate of DO for study of the urodynamic changes that occur after bladder outflow obstruction/deobstruction. In this study, therefore, we focused on this parameter.

The occurrence and frequency of NVCs may be influenced by several factors. One is the surgical trauma of the repeated abdominal or urethral surgery involved in the conventional experimental approach. We tried to reduce this factor by introducing a modified experimental procedure, which not only increased the survival rate after deobstruction but also resulted in urodynamic findings differing from those previously reported after deobstruction. We also used simultaneous registrations of IVP and IAP to record true detrusor activity. The animals were kept obstructed for 2 wk, because it is known that bladder function stabilizes by 2 wk after the initiation of partial outlet obstruction (22). In our study, the deobstructed animals were studied 1 wk after deobstruction. This may be a short time period for recovery; nevertheless, 60% of the animals studied by our modified approach had normalized bladder function without NVCs.

Rats obstructed by means of our approach showed a significant increase in bladder weight, pressure parameters (including BP and TP), volume parameters (including BC and RV), and compliance after obstruction. These findings are consistent with published results from animal models in which obstruction was performed by means of conventional methods (20, 23, 24, 27, 30). In those studies, obstruction was most often produced by placing one or two loose ligatures around the proximal urethra with a rod of a specific diameter that was then

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Fig. 5. Representative light micrographs of a thin section of detrusor (A) and the % smooth muscle or collagen over the total sum (B) in sham animals, rats obstructed by means of our modified method (Modif-Obs), deobstructed rats showing NVCs (DO+), and deobstructed rats showing no NVCs (DO−). Scale bar = 100 μm. *P < 0.05; ***P < 0.001.
removed to avoid consequent ischemic muscular damage. However, the urethra is apt to be injured during the dissection between the urethra and the vagina and may rupture because the posterior part of the friable urethra is tightly attached to the vaginal epithelium. To solve this problem, we tied a 3-0 nylon suture loosely around the urethra, including the adjacent vaginal epithelium, which may have had a cushioning effect on the compression. We speculate that the obstructing mechanism of this approach is to kink the urethra rather than compress it. By use of this approach, we could avoid one abdominal incision.

We found that in the modified obstruction/deobstruction (Modif-Obs/Deobs) group, 40% of the animals showed NVCs during the filling phase, as verified by the simultaneous measurements of IAP and IVP. This incidence is similar to what is found clinically. In contrast, all rats in the conventional obstruction/deobstruction (Conv-Obs/Deobs) group exhibited NVCs. The Conv-Obs/Deobs group showed high bladder pressures (BP and TP) and high volume parameters (BC and MV) compared with the sham group. The C-Obs/Deobs group also showed lower compliance than did the Modif-Obs and Modif-Obs/Deobs groups.

There was no significant difference in mortality after the obstruction procedure in the Modif-Obs, Modif-Obs/Deobs, and Conv-Obs/Deobs groups. However, after deligation, the Conv-Obs/Deobs group showed a 25% mortality rate, compared with no mortality in the Modif-Obs/Deobs group. Mortality in the Conv-Obs/Deobs group was similar to that previously reported by Chai et al. (6). Both the differences in mortality and the differences in function between the Conv-Obs/Deobs and Modif-Obs/Deobs groups might be consequences of the less extensive surgery in the Modif-Obs/Deobs animals.

Previous studies (37, 38) showed that obstruction increases the total amount of bladder collagen, but that, due to the increase in bladder mass, the concentration decreases significantly. We confirmed a decrease in the percentage of collagen after obstruction/deobstruction in all groups, except the deobstructed DO− group. These changes are probably caused by increased functional demands by BOO on the bladder, such as...
an increase in tension or strain. DO+ deligated rats may have functional BOO, possibly as the result of persistent changes in the central regulation (6). The deobstructed DO− group showed values closer to those of the sham group than the obstructed group. This structural normalization may be related to the absence of NVCs.

Chai et al. (6) showed persistent hyperactive voiding after relief of obstruction in 20% of rats. They defined hyperactive voiding on the basis of voiding frequency, using a 4-h period during the light cycle in a metabolic cage without cystometry. In their urodynamic investigation, which was not presented in detail, they found that hyperactive voiders had significantly higher micturition pressures than did normalized voiders and that the volume of urine per void was lower. This agrees with the findings in our DO+ group.

Possible causes for storage symptoms after deobstructive surgery have been suggested, including persistent obstruction (3, 5, 6, 13, 36). In our study, persistent DO could not be attributed to persistent obstruction, because the percentage of RV to BC was significantly decreased from 36% in the Modif-Obs group to 7% in the Modif-Obs/Deobs group. We found that the bladder weight was not decreased after deobstruction, which suggests that the bladder changes during obstruction were not completely reversible. Furthermore, in the Modif-Obs/Deobs group, the DO+ animals showed a higher ratio than did the DO− subgroup, which may be related to the presence of increased detrusor contractions during the filling phase. Even after relief of obstruction, the changed bladder may not return to its original state before obstruction, and this might result in remaining symptoms (35). For example, Kojima et al. (17) showed that their patients with a high ultrasound-estimated bladder weight above the fixed level of 80 g were characterized by irreversible pathological changes in the detrusor.

Measurement of IAP is standard in human cystometry (31) and provides information on the true DP. However, use of this measurement is still limited in animal studies. Its advantage is that nonvoiding detrusor contractions can be discriminated from abdominal straining during the filling phase (16, 20). With the presence of simultaneous changes on the IVP curve and the IAP curve, we classified the rats as those with or without NVCs. The NVCs were found in 80% of the Modif-Obs group, in 40% of the Modif-Obs/Deobs group, and in 100% of the Conv-Obs/Deobs group. The characteristics of the NVCs were significantly different among the three groups with regard to frequency and pressure. The Modif-Obs/Deobs group showed a lower frequency of DO than did the Conv-Obs/Deobs group and a lower pressure amplitude of NVCs than was found in the Modif-Obs group.

In conclusion, in rats deobstructed by means of conventional methods, we observed changes in bladder function and NVC characteristics that may be related to the surgical morbidity and mortality resulting from multiple operations. In contrast, rats operated on by means of our modified approach showed urodynamic changes similar to those found in clinical conditions. We confirmed that a significant proportion of deobstructed rats showed a normalization of both structural and functional parameters. In animals with persistent NVCs after deligation, the obstruction may have caused irreversible or
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slowly reversible changes in bladder structures (e.g., gap junctions) (7) causing these functional abnormalities.

Perspectives and Significance

Our comparison of the conventional method with our new method of partial BOO in female rats, which can subsequently be relieved through the vagina, demonstrates that surgical morbidity resulting from multiple, accumulating, operational hazards can influence the changes in bladder structure and function caused by outflow obstruction and deligation. With the use of our new method, some of these hazards can be avoided. This change in methodology will make it possible to more accurately analyze the molecular mechanisms responsible for the changes observed after obstruction and deligation.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


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