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Restoration of rat bladder function following release of short- and long-term partial outflow obstruction

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Abstract Detrusor dysfunction does not recover in some patients with benign prostatic hyperplasia (BPH) even after prostate resection. 'We studied the functional restoration of the rat bladder after release of short- or long-term outflow obstruction. Bladder function was assessed by in vivo infusion cystometry and an in vitro organ bath technique. There were no significant differences in bladder weight and contractile strength induced by stimuli in detrusor muscle strips from obstructed rats and age-matched control rats. After short-term obstruction the whole bladder pressure generated in vitro by field stimulation, bethanechol, ATP, and KCl completely recovered to control levels. In contrast, after long-term obstruction, the whole bladder pressure in response to field stimulation remained significantly lower than in controls. Infusion cystometry variables, including the pressure at which micturition was induced, maximal voiding pressure, capacity, and residual urine volume, were similar between controls and rats subjected to short-term obstruction. However, the maximal voiding pressure after long-term obstruction was significantly less than that of controls.

Key words Release of outflow obstruction · Rat bladder · Detrusor function · Benign prostatic hyperplasia

Introduction

Recovery of detrusor dysfunction following surgical resection of the prostate is controversial [4, 20, 21]. After prostatectomy, about one-third of patients complain of bladder irritability resulting in urgency and/or urge incontinence, while other patients suffer from a decreased urine stream because of impaired detrusor contractility.

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Symptoms of outflow obstruction secondary to benign prostatic hyperplasia (BPH) can be reproduced in basic animal models [1–3, 5, 6, 12, 14–16]. Previous studies from our laboratory have shown that the detrusor contractile response to various stimuli is significantly increased by short-term outflow obstruction of up to 28 days, with an increase in nocturnal micturition frequency [14]. In contrast, longer periods of obstruction, e.g., 3–6 months, significantly impair detrusor contractility with over 60% of rats voiding urine with overflow-type incontinence [15]. In this study, we assessed bladder function in rats following release of either short-term or long-term obstruction.

Materials and methods

Operative procedure

Twelve-week-old male Sprague-Dawley rats (Chubu Kagaku Inc., Aichi Japan) (300–350 g) were anesthetized with sodium pentobarbital (50 mg/kg). Sterile technique was employed for the surgical creation of a partial outflow obstruction. Supine rats underwent a midline suprapubic incision, and the prostate lobes were retracted to expose the bladder neck and urethra without causing damage to the bladder. Bilateral dissection of the space surrounded by the ureter, the urethra, and the vas deferens was performed. After placement of a catheter (outside diameter 1.70 mm) on the urethra, a 2-0 silk suture was passed behind the urethra and ligated. The catheter was then removed. The presence of the catheter ensured that the ligature did not significantly compress the urethra. The bladder and prostate were returned to their normal positions and the skin incision was closed. Control rats of the same age were maintained under the same conditions but did not undergo surgery. The urethral ligation was removed through the same skin incision under pentobarbital anesthesia. Experimental animals were divided into two groups. Group A consisted of 12 rats with urethral obstruction for 2 weeks, with assessment of bladder function 4 weeks after release of the obstruction. Group B was composed of 12 rats subjected to urethral obstruction for 6 months, with assessment 3 months after the obstruction was released. Twelve age-matched control rats were used for group A and ten for group B.

In vivo infusion cystometry

Anesthetized rats (urethane, 1.2 g/kg) were placed in a supine position and a suprapubic longitudinal incision was made. The

bladder was gently exposed, and intubated suprapubically with a double-lumen catheter which was then ligated with a 4-0 silk suture. The ureters were not ligated. The outside catheter (OD 1.20 mm) was connected to a pressure transducer, and the intravesicular pressure was recorded continuously on a Rectigraph 8S (San-ei Co., Tokyo, Japan). The inner catheter (OD 0.54 mm) was connected to an infusion pump (model STC-521, Terumo Co. Tokyo, Japan) and saline was infused. To eliminate the effect of infusion pressure on intravesical pressure, the inner catheter was 2 mm longer than the outside catheter.

The infusion rate was set at 0.05 ml/min. Capacity, maximal voiding pressure, pressure at which micturition was induced, voided urine volume, and residual urine volume were measured in obstruction-released and control rats. Capacity was defined as the volume of voided plus residual urine at voiding. Voiding pressure was defined as the point of phasic increase in bladder pressure which resulted in voiding. The pressure at which micturition was induced was the intravesical pressure just before the phasic contraction for voiding started.

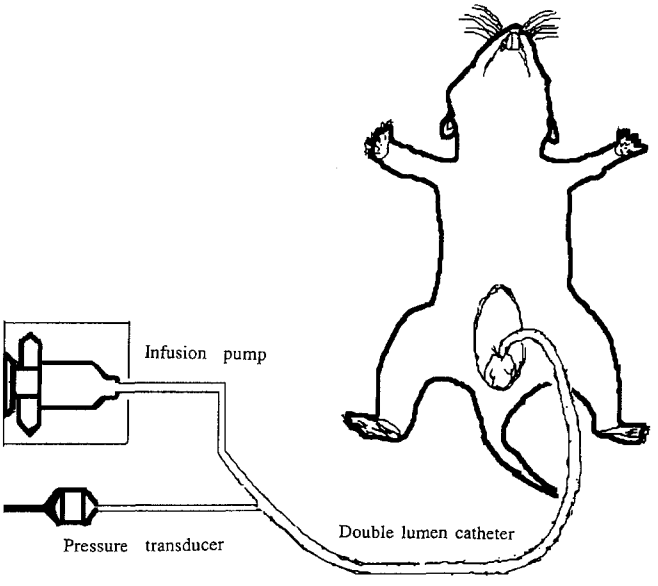


Fig. 1 Experimental setup for in vivo rat infusion cystometry

In vitro whole bladder studies

After infusion cystometry, the ureters and urethra were ligated with 3-0 silk sutures. The entire bladder was excised and transferred into an organ bath containing 30 ml Krebs' solution (NaCl 119 mM, KCl 4.7 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.2 mM, $NaHCO_3$ 25 mM, $CaCl_2$ 2.5 mM, and glucose 11 mM). The bladder was filled to a capacity of 0.5 ml and incubated for 30 min under an atmosphere of 95% O_2 and 5% CO_2. Pressure increases generated by field stimulation, bethanechol (7.4 and 600 μM), ATP (2 mM), and KCl (124 mM) were measured. Field stimulation utilized platinum electrodes set on both sides of the tissue in the organ bath. Transmural nerve stimulation was performed with a DPS-160 field stimulator (Dia Medical System, Tokyo, Japan) delivering biphasic square wave pulses of 50 V, 1 ms duration, and variable frequencies (2, 4, 8, 16, 30, and 60 Hz). Stimulation was maintained for 5 s. The interval between stimulations was 2 min. The polarity of electrodes was changed after each pulse by means of a polarity-changing unit. Contraction of the bladder to field stimulation was almost completely suppressed by 10^{-6} M tetrodotoxin.

In-vitro study of muscle strips

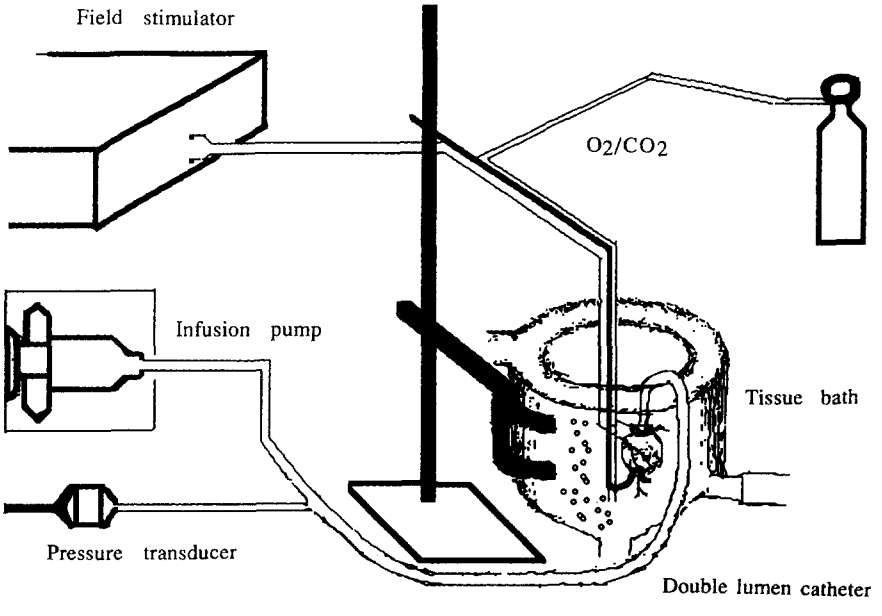
After bladder weight had been recorded, two longitudinal strips (8 × 1.5 mm) were created from the bladder body. These muscle strips were suspended in an individual organ bath containing 10 ml Krebs' solution at 37 °C under an atmosphere of 95% oxygen and 5% carbon dioxide. One end of each strip was connected to a force displacement transducer (Model 45196 San-ei Co., Tokyo, Japan) and changes in muscle tension were measured and recorded on a Rectigraph-8 K (San-ei Co., Tokyo, Japan).

Each strip was equilibrated for 30 min. Resting tension was adjusted to approximately 1 g at the end of incubation. Tension increases in response to field stimulation, bethanechol at concentrations ranging from 0.8 to 600 μM, and maximal doses of ATP (2 mM) and KCl (124 mM) were determined under the same experimental conditions as the whole bladder study.

Drugs

Bethanechol, tetrodotoxin, and ATP were obtained from Sigma, St. Louis, MO. A high potassium solution was prepared by replacing NaCl with an equimolar amount of KCl in Krebs' solution.

Fig. 2 Experimental setup for whole rat bladder in vitro



Statistical analysis

Measurements in infusion cystometry and pressure increases in in vitro whole bladder studies were expressed as cmH_2O. The in vitro contractile strength of muscle strips was normalized by tissue weight (g/100 mg tissue). Statistical analysis utilized Fishers' protected least significant difference test. A probability level of $P < 0.05$ was accepted as statistically significant.

Results

Mean bladder weight was not different between the two experimental groups and the respective age-matched controls: 146.4 ± 4.1 and 141.0 ± 8.2 mg for group A and controls, respectively, and 183.0 ± 11.3 and 173.8 ± 5.6 mg for group B and controls, respectively. Values for each cystometric measurement are shown in Table 1. In group A there was no difference in means for cystometric variables compared to controls. However, the mean maximal voiding pressure for group B rats was significantly lower than for controls. A representative cystometrogram of group B and a control are illustrated in Fig. 3. In the in vitro whole bladder model, the responses of group A rats to field stimulation, bethanechol, ATP, and KCl were not altered compared with controls. However, the mean whole bladder pressure generation for group B rats in response to high freqency of field stimulation was significantly weaker than for controls (Table 2, Fig. 4). In vitro tension increases of muscle strips in response to field stimulation, bethanechol, ATP, and KCl were not different between the two experimental groups and the respective age-matched controls (Table 3).

Discussion

Pathological detrusor function in the patients suffering from BPH has been a long-standing subject of various experimental and clinical studies [1–3, 5, 6, 12, 14–16, 21]. Bladder symptom secondary to outflow obstruction can be categorized into two groups: irritative symptoms such as urgency or urge incontinence influenced by detrusor hyperactivity, and obstructive symptoms of weak stream or incomplete evacuation caused by impaired detrusor contractility. Animal experiments have suggested that the partial outflow obstruction for a short

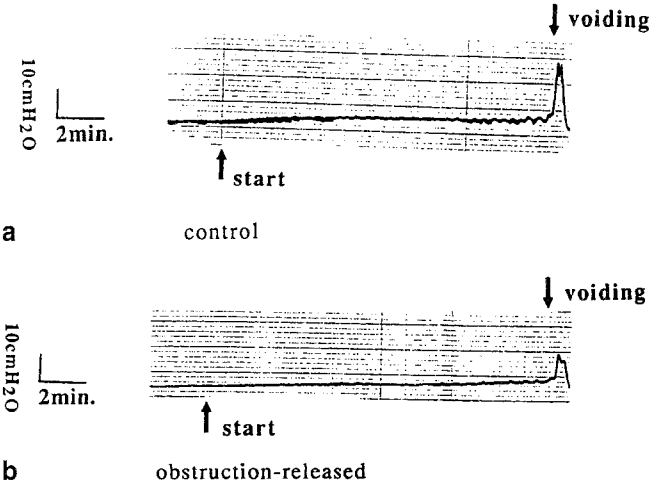


Fig. 3a, b A representative cystometrogram from a group b bladder (**b**) and an age-matched control (**a**)

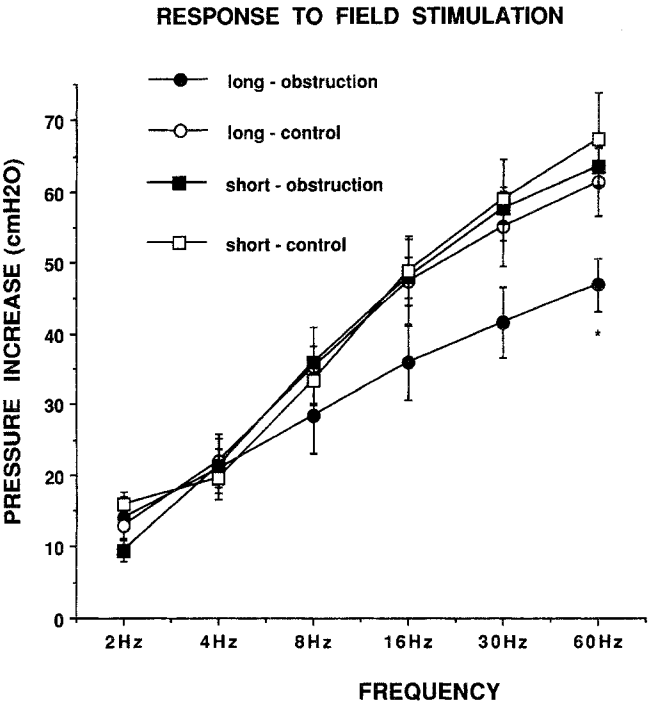


Fig. 4 Frequency response curves. Each point represents a mean ± SEM of six (short-obstruction and short-control) or four (long-obstruction and long-control) individual observations. *Significant difference from the value of age-matched controls, $P < 0.05$

Table 1 Mean values of cystometric variables in obstruction-released and control bladders

Parameter	Group A ($n = 6$)	Control ($n = 6$)	Group B ($n = 10$)	Control ($n = 6$)
Pressure at which micturition was induced (cmH_2O)	4.6 ± 0.84	5.7 ± 0.51	6.3 ± 0.76	4.8 ± 0.86
Bladder capacity (ml)	0.45 ± 0.08	0.41 ± 0.06	0.59 ± 0.14	0.57 ± 0.13
Maximum voiding pressure (cmH_2O)	21.3 ± 2.2	22.8 ± 2.7	12.4* ± 1.5	20.2 ± 1.2
Volume of residual urine (ml)	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.02	0.01 ± 0.01

*Significant difference from control bladder, $P < 0.05$

Table 2 Mean maximum pressure increases of rat whole bladders generated in vitro in response to various stimuli (cmH_2O)

Stimulations	Group A ($n = 6$)	Control ($n = 6$)	Group B ($n = 4$)	Control ($n = 4$)
Field stimulation (60 Hz)	63.3 ±2.8	67.3 6.4	46.3* ±3.8	61.3 ±4.7
Bethanechol (600 μM)	53.5 ±2.9	61.2 ±5.6	51.5 ±6.7	59.5 ±4.9
ATP (2 mM)	10.2 ±1.1.	11.8 ±2.3	13.0 ±3.8	13.0 ±1.3
KCl (124 mM)	43.0 ±1.9	51.2 ±3.4	44.0 ±4.7	48.3 ±2.8

* Significant difference from control bladder, $P < 0.05$

Table 3 Mean maximal tension increases in muscle strips taken from obstruction-released and control bladders

Stimuli	Group A ($n = 6$)	Control ($n = 6$)	Group B ($n = 6$)	Control ($n = 6$)
Field stimulation (60 Hz)	36.1 ±2.6	34.9 ±2.7	30.9 ±2.9	32.6 ±3.0
Bethanechol (600 μM)	46.4 ±3.1	46.2 ±2.6	37.5 ±3.0	37.8 ±2.6
ATP (2 mM)	6.9 ±0.6	7.0 ±0.9	5.6 ±0.8	6.9 ±1.2
KCl (124 mM)	37.8 ±2.9	36.1 ±2.0	29.5 ±2.8	31.2 ±2.7

period of time results in increased detrusor contractility [5, 6, 14, 18], but that severe obstruction lasting for a long time impairs the detrusor contractility [3, 6, 12, 15, 16]. These basic findings are not always applicable to clinical situations because the time of onset of obstruction is usually unknown and other etiologies for detrusor dysfunction may be involved.

Our previous study demonstrated that partial urethral ligation of up to 28 days in rats increases the nocturnal frequency of micturition in vivo and augments detrusor contractility in vitro [14]. The present study showed that release of outflow obstruction lasting 2 weeks returned bladder contractility to normal in vivo and in vitro, which is consistent with the results of Speakman et al. [18]. Using pigs they demonstrated that increased detrusor responses to acetylcholine, KCl, and field stimulation following 3 months of obstruction were returned to normal by releasing the obstruction. These findings suggest that compensatory mechanisms involving detrusor muscle play a role in overcoming the increased flow resistance caused by obstruction so that elimination of outflow obstruction normalizes detrusor activity. Clinical observations suggest that resection of the prostate would be beneficial for patients having detrusor instability due to BPH [21].

Using rats subjected to long-term obstruction, we have previously demonstrated that detrusor contractility decreases in a time-dependent fashion and that over 60% of rats so treated evacuate urine by overflow incontinence [15]. The current study, however, showed that 3 months after release of a 6-month-long obstruction, detrusor function as well as bladder weight recovered almost to normal levels except for two variables: maximal voiding pressure and the contractile force of the whole bladder in response to field stimulation. A decrease in voiding pressure in vivo can be induced by a decrease in urethral resistance. However, since whole bladder pressure generation in vitro was also suppressed in long-term obstructed bladder, it is possible that nerve-mediated responses may be slow to recover compared to other physiologic bladder processes involving direct receptor stimulation or direct muscle stimulation. In the past some reports have suggested that decreased detrusor contractility can be fully restored after release of obstruction [7, 10, 11], but other studies have found that this is not so [17, 18]. Seki et al. [17] have shown that some electrical activities of the detrusor muscle membrane are irreversibly affected even after obstruction is relieved. Steers et al. [19] have found that releasing obstruction is not enough to restore morphological normalcy in the major pelvic ganglion; however, Gabella et al. have shown complete recovery of this structure [2].

It should be noted that 3 months after release of 6-month obstruction response of muscle strip to field stimulation was completely recovered; however, pressure generation of whole bladder to field stimulation in vitro remained significantly lower than in age-matched controls. We should recognize that the response of smooth muscle strip and whole bladder is not always the same, for instance, the response of whole bladder to β-adrenergic stimulation is substantially smaller than that of muscle strip [8]. The discrepancy demonstrated in this study suggested that the integration of the nervous system in the bladder may be restored slowly.

Acute pharmacological study indicated that the severity of outflow obstruction used in this study is milder than that found by other studies [3, 5–7, 12], because of a small increase in bladder weight and a significant increase in the contractile strength of muscle strips to stimuli [14, 16]. However, it has been found that when the duration of obstruction becomes longer, the contractility decreases in a time-dependent manner with a larger increase in bladder weight [15]. At 6 months after obstruction the contractile strength decreased significantly with a six-fold increase in bladder weight. Both contractility and bladder weight recovered after release of obstruction. The decrease in bladder mass after release of obstruction is consistent with that found by other reports [10, 11, 17, 18]. However, the rate of recovery of bladder weight differs between reports, probably depending on severity and/or duration of outflow obstruction, and the period following release of obstruction at which the examination was performed.

For patients with BPH showing a weak stream and a large amount of residual urine volume, prediction of bladder recovery often requires a pressure-flow study. It is generally believed that those outflow-obstructed patients having a strong increase in detrusor pressure will

be ideal candidates for prostate surgery but that those with weak detrusor pressure at voiding will not benefit from surgery [9, 13]. Our previous [15] and present animal studies, however, suggest that the impaired detrusor activities caused by long-term obstruction do sometimes recover to almost normal levels after release of obstruction. Further studies are necessary to resolve the inconsistency between clinical observation and the results of animal studies.

In summary, release of outflow obstruction lasting for 2 weeks completely restored bladder function 4 weeks later. However, relief of obstruction lasting for 6 months failed to fully reverse abnormalities in 2 of the 13 variables measured 3 months later, i.e., the maximal voiding pressure in vivo and the whole bladder pressure generated in vitro in response to field stimulation.

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