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Functional restoration of rat bladder after subtotal cystectomy: in vivo cystometry and in vitro study of whole bladder

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Abstract Functional restoration of the rat urinary bladder following subtotal cystectomy was studied via in vivo infusion cystometry and an in vitro whole bladder model. After the bladder had been separated from the prostate, subtotal cystectomy was achieved by ligating the bladder completely at a level just above the insertion of the ureters into the bladder. Bladder function was investigated immediately and 7, 14, and 28 days after surgery. Bladder weight was reduced to 17% that in sham-operated controls immediately after surgery, but recovered to 76% of that in controls 28 days after the operation. In vivo capacity also increased after surgery from 13% that of controls to 59% 28 days later. However, voiding pressure remained low (34% of control) 28 days later. An in vitro whole bladder study showed that the response to field stimulation decreased significantly on day 7, but had recovered considerably by day 28. The maximal response to bethanechol decreased significantly 7 days after surgery, but recovered thereafter. The response to phenylephrine increased significantly immediately after surgery, but gradually returned to the control level. An in vitro volume-pressure study showed that passive compliance of the cystectomized bladder decreased after surgery, but improved with time. The peak of the active pressure increase to field stimulation occurred at a low infusion volume immediately after surgery, but bladder capacity increased gradually until 28 days later, when the maximal active pressure was obtained. Our results suggest that restoration of the bladder following subtotal cystectomy may not derive simply from an expansion of the bladder wall. Functional alteration involving the bladder base was also observed.

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Introduction

The issue of regeneration or expansion of the urinary bladder after subtotal cystectomy is controversial [2, 4–9]. Although bladder capacity increases with time after subtotal cystectomy [6, 7, 10], functional restoration is not certain [8]. Distribution of autonomic receptors in the bladder base is different from that in the bladder body. The principal efferent peripheral neurotransmitter is acetylcholine for the bladder body (muscarinic-cholinergic) and norepinephrine for the bladder base and urethra (α-adrenergic). Voiding is initiated and completed by stimulation of cholinergic receptors in the bladder body and by suppression of α-adrenergic receptors in the bladder base and urethra. Since subtotal cystectomy removes most of the bladder body, postoperative bladder function should be different from that of the control bladder. We report here changes in function of the rat urinary bladder after subtotal cystectomy, as studied via in vivo infusion cystometry and an in vitro whole bladder model.

Materials and methods

Operative procedures

Male Sprague-Dawley rats (350–400 g) purchased from Chubu Kagaku were anesthetized with sodium pentobarbital (50 mg/kg), and the bladder and prostate were exposed through a lower abdominal incision. The bladder was separated from the prostate and drained of urine by cutting the bladder dome. Subtotal cystectomy was done by ligating the bladder completely with 2-0 silk suture at a level just above the insertion of the ureters into the bladder. In a sham operation, the bladder was separated from the prostate; these rats were used in experiments 14 days after surgery. Bladder function

Table 1 Effects of subtotal cystectomy on bladder weight and the parameters of in vivo infusion cystometry. Means ± SEM of five or six individual studies

Parameter	Sham-operated control	Time after subtotal cystectomy			
		Immediately	7 days	14 days	28 days
Bladder weight (mg)	111.4 ± 23.7	19.2* ± 1.3	44.9* ± 2.14	62.3 ± 3.3	84.8 ± 5.6
Bladder capacity (ml)	0.46 ± 0.11	0.06* ± 0.01	0.16* ± 0.02	0.17* ± 0.02	0.27 ± 0.03
Voiding pressure (cmH_2O)	29.8 ± 2.6	5.5* ± 0.7	5.5* ± 0.8	8.3* ± 0.9	10.2* ± 1.2
Pressure at which micturition was induced (cmH_2O)	3.5 ± 0.6	6.3* ± 1.0	5.7 ± 1.2	4.7 ± 0.7	4.7 ± 0.9
Residual urine (ml)	0.05 ± 0.03	0.04 ± 0.01	0.12 ± 0.02	0.04 ± 0.02	0.14 ± 0.03

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

was compared with that of sham-operated rats immediately and 7, 14, and 28 days after subtotal cystectomy.

In vivo infusion cystometry

Under urethane anesthesia (1.2 g/kg), to expose the bladder without damage, the bilateral prostate was retracted through a lower abdominal incision. After a double-lumen catheter had been intubated suprapubically, the catheter and bladder wall were ligated with 2-0 silk suture. The prostate and bladder were returned to normal position and infusion cystometry was carried out. The outside catheter [outside diameter (OD) = 1.20 mm] was connected to a pressure transducer and the increase in intravesical pressure was monitored continuously on a Rectigraph 8S (San-ei, Tokyo, Japan). The inner catheter (OD = 0.61 mm) was connected to an infusion pump (Model STC-521, Terumo, Tokyo, Japan) and the rate of saline infusion was set at 0.05 ml/min. Capacity, voiding pressure, the pressure at which micturition was induced, and residual urine volume were measured. To eliminate any effect of infusion pressure on intravesical pressure, the inner catheter was 2 mm longer than the outside catheter.

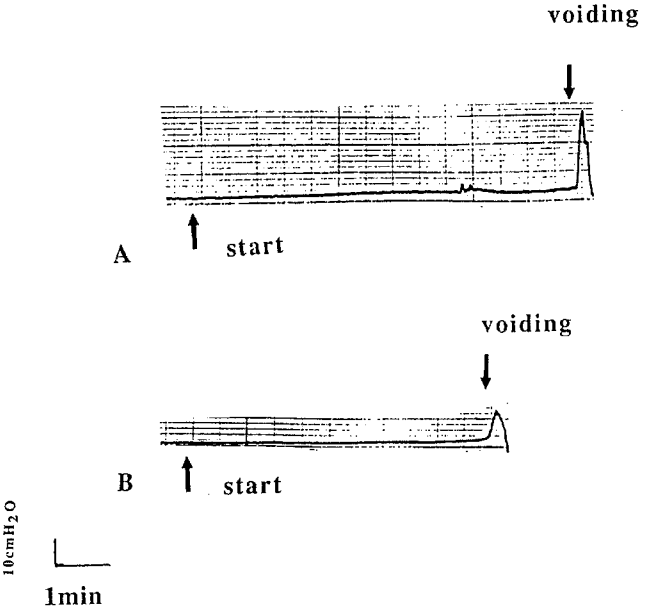

Fig. 1A, B Representative tracings of infusion cystometry. **A** Sham-operated controls. **B** 28 days after subtotal cystectomy

In vitro whole bladder study

After the in vivo study, the ureters and urethra were ligated with 3-0 silk suture. The entire bladder was excised and transferred to 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, $CaCl_2$ 2.5 mM, $NaHCO_3$ 25 mM and glucose 11 mM). Under each bladder capacity, which was determined by in vivo cystometry (Table 1), the bladder was incubated for 30 min with a mixture of 95% O_2 and 5% CO_2. Pressure increases generated by field stimulation, bethanechol (7.4 and 600 μM), ATP (2 mM), phenylephrine (200 μM), and KCl (124 mM) were measured. Field stimulation utilized platinum electrodes set on both sides of the muscle strips in the organ bath. Transmural nerve stimulation was applied with a DPS-160 field stimulator (Dia-Medical System, Tokyo, Japan) that delivered biphasic square-wave pulses of 50 v, 0.5 ms in duration; the interval between stimulations was 2 min. Contraction of the bladder to field stimulation was almost completely suppressed by 10^{-6} M tetrodotoxin. A frequency-response study to frequencies ranging from 2 to 60 Hz was done. A high-potassium solution was prepared by replacing NaCl with an equimolar amount of KCl in Krebs' solution.

Finally, a volume-pressure study was performed as follows: After intravesical saline had been evacuated completely, the bladder was incubated for 30 min to determine the zero intravesical pressure and was then stimulated with 30 Hz field stimulation. Subsequently, saline was instilled at a rate of 0.05 ml/min. Field stimulation was repeated at 2-min intervals. Passive pressure defined as the intravesical pressure just before stimulation was determined. The active pressure increase in response to field stimulation was defined as peak pressure at stimulation minus passive pressure.

Drugs and statistics

Bethanechol, ATP, and phenylephrine were purchased from Sigma. Data are expressed as means ± SEM, except for the results of the volume-pressure study, which are illustrated with only one mean to simplify the graphs. Statistical analysis was done using the unpaired Student's t-test. A level of $P < 0.05$ was accepted as being statistically significant.

Results

Bladder weight and parameters from the in vivo cystometrograms are shown in Table 1. Bladder weight was significantly reduced immediately after subtotal

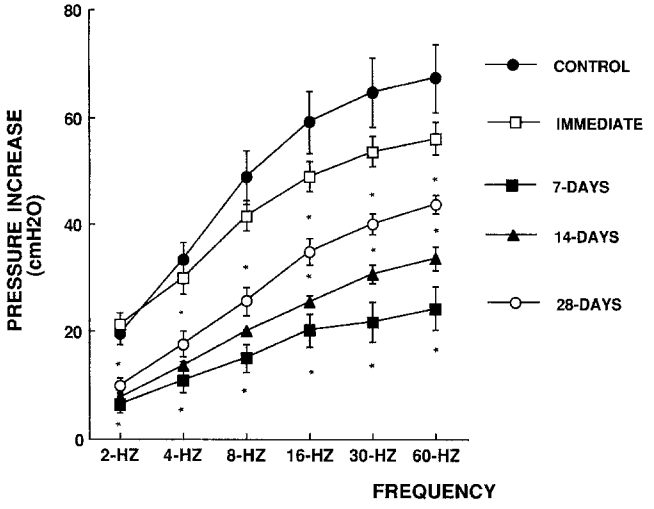

Fig. 2 Effects of subtotal cystectomy on the response to field stimulation. *Each point* is the mean ± SEM of five or six individual observations. * Significant difference from control value, $P < 0.05$

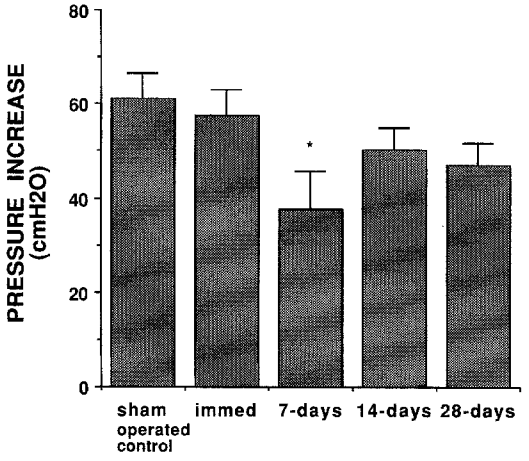

Fig. 3 Effects of subtotal cystectomy on the response to a high concentration of bethanechol (600 μM). *Each bar* is the mean ± SEM of five or six individual observations. * Significant difference from control value, $P < 0.05$

cystectomy, but recovered to 76% of that observed in the sham-operated controls 28 days after operation. In vivo capacity returned to 59% of the control value after 28 days. However, voiding pressure remained low (34% of control) 28 days later. Immediately after surgery, the pressure at which micturition was induced increased significantly, as compared with controls. Representative cystometrograms of the sham-operated control and of a cystectomized bladder are shown in Fig. 1. An in vitro whole bladder study showed that the response to field stimulation was significantly decreased on day 7, but had recovered considerably by day 28 (Fig. 2). The maximal response to bethanechol decreased significantly 7 days after subtotal cystectomy, but had been restored to normal by day 14 (Fig. 3). Responses to

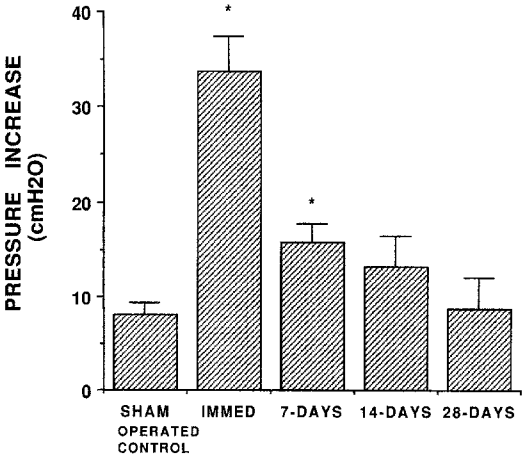

Fig. 4 Effects of subtotal cystectomy on the response to phenylephrine. *Each bar* is the mean ± SEM of five or six individual observations. * Significant difference from control value, $P < 0.05$

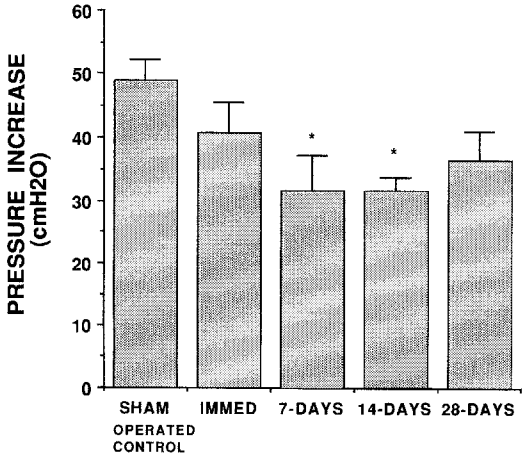

Fig. 5 Effects of subtotal cystectomy on the response to KCl. *Each bar* is the mean ± SEM of five or six individual observations. * Significant difference from control value, $P < 0.05$

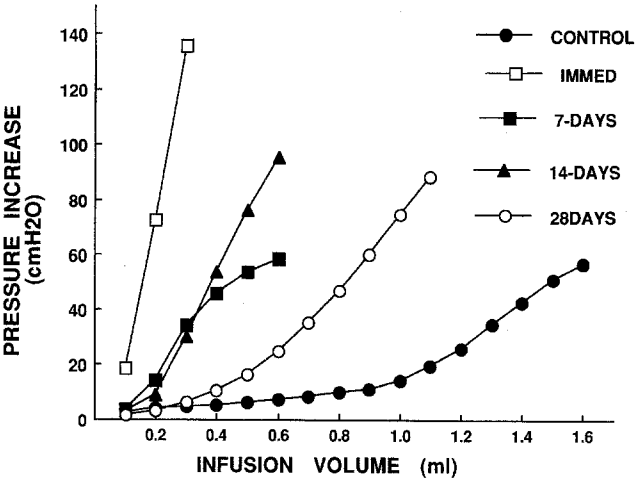

Fig. 6 Effects of subtotal cystectomy on the in vitro passive pressure increase determined as an increase in intravesical volume. *Each bar* is the mean of five or six individual observations

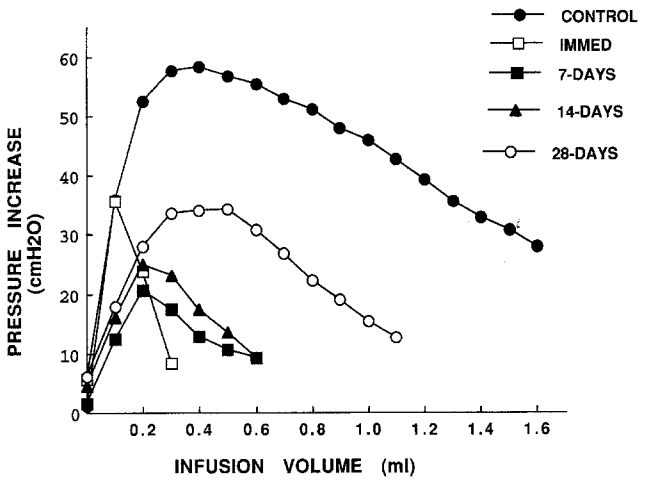

Fig. 7 Effects of subtotal cystectomy on the in vitro active pressure increase in response to field stimulation, determined as an increase in intravesical volume. *Each bar* is the mean of five or six individual observations

a low concentration of bethanechol and ATP were not affected by surgery (data not shown). The pressure increase in response to phenylephrine was significantly augmented immediately after surgery, but gradually returned to the control level within 14 days (Fig. 4). A significant decrease in the response to KCl was observed on days 7 and 14 (Fig. 5).

The passive volume-pressure study showed that in vitro bladder compliance decreased immediately after surgery, but improved with time (Fig. 6). The peak of the active pressure increase to field stimulation occurred at a low infusion volume immediately after surgery, but bladder capacity increased gradually to the point at which a maximal active pressure response was obtained (Fig. 7).

Discussion

Regeneration is generally defined as the reiterated formation of a lost part or parts of a morphological unit, or the new formation of lost cells from cells of the surrounding tissue [10]. After a subtotal cystectomy, in which most of the entire bladder body is resected surgically, the capacity and mass of the bladder reportedly recover to the presurgical level within a few months [6, 7, 10]. The mechanism of the functional restoration remains to be clarified [10]. Voiding is initiated and completed by stimulation of cholinergic receptors in the bladder body, and urine storage is maintained by stimulation of α- and β-adrenergic receptors in the bladder.

The rat bladder has a very sparse supply of adrenergic nerves except in the trigonum area, where it has a much richer supply. Furthermore, in rat there is a rich supply of cholinergic nerves in all parts of the bladder [1, 3]. Alm and Elmer have shown no apparent differences in the number and distribution of acetylcholine esterase positive nerves between bladder body and bladder neck [1]. Subtotal cystectomy greatly reduces the bladder body mass and inevitably alters the ratio of autonomic receptors in the remaining bladder. Therefore, it is reasonable to assume that the cystectomized bladder is governed primarily by α-adrenergic receptors. The response to α-adrenergic stimulation increased significantly immediately after surgery, which seemed to reflect this alteration, but the response subsequently returned to the level of the sham-operated control bladder. The fact that the cystectomized bladder immediately after surgery contracted in response to bethanechol as strongly as did the control can be explained by the findings that the rat bladder has a rich supply of cholinergic nerves even in the bladder neck [1, 3].

Immediately after surgery, the pressure at which micturition was induced in vivo increased significantly, which helped the bladder wall to expand. Bladder weight and capacity increased over the next 7 days, but the in vitro contractility in response to bethanechol decreased. These changes may be provoked by the rapid distention of the bladder wall that, in turn, damages smooth muscle cells and decreases detrusor contractility. Finally, 28 days after surgery, the responses of the cystectomized bladder to bethanechol, phenylephrine, ATP, and KCl were all similar to control values, suggesting that the rat bladder base had the ability to change its pharmacologic responses in so short time. This was not the case in the rabbit bladder [8], for Lin et al. reported that even 8 weeks after surgery the cystectomized bladder still showed an increased response to α-adrenergic stimulation and a decreased response to cholinergic stimulation, as compared with control bladder. Species differences will account for this disparity.

The bladder volume that produced the maximal pressure response to field stimulation in the in vitro active volume-pressure study was closely related to the maximum bladder capacity in vivo. This finding suggests that at the maximum bladder capacity in vivo the bladder can work with greatest power to evacuate urine.

In summary we observed that the function of the rat bladder in vivo returned to near-normal level 28 days after a subtotal cystectomy that had greatly altered all bladder functions immediately after surgery. These findings suggest that the restoration of the bladder parameters involves not only distention of the bladder wall but also a functional alteration involving the base of bladder.

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