EFFECTS OF *IN VIVO* ISCHEMIA ON THE INFUSION CYSTOMETRY AND *IN VITRO* WHOLE BLADDER CONTRACTILITY OF THE RAT

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Ischemia induced by atherosclerosis is a common cause of organ failure in the elderly. We investigated the effects of *in vivo* ischemia created by ligation of the internal iliac arteries on the parameters of *in vivo* infusion cystometry under urethane anesthesia and on *in vitro* whole bladder contractility of the rat.

Bladder weight significantly increased after ischemia for 14 days. Infusion cystometry demonstrated that in the ischemic bladders the capacity increased, the voiding pressure decreased, and the volume of residual urine increased, which resulted in deteriorated voiding efficacy. The *in vitro* whole bladder contractility to field stimulation, bethanechol, ATP, and KCl was reduced by ischemia. The passive pressure increased as the bladder volume enlarged and the bladder compliance once decreased by ischemia on the 7th day, but increased on the 14th day. In an active volume-pressure relationship study the peak response was decreased by ischemia. The volume at which response reached a peak value shifted to a larger volume 14 days after surgery.

In conclusion, ischemia impaired *in vivo* rat detrusor power to empty. Since detrusor contractility *in vitro* decreased in response to various kinds of stimulation, this deteriorated bladder function was supposed to be caused by muscle degeneration.

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Key words : Ischemia, Rat bladder function, Infusion cystometry, *In vitro* whole bladder

INTRODUCTION

Atherosclerosis with aging causes ischemia and deteriorates the function of various organs. Normal function of the urinary bladder is dependent on the adequate blood flow which supplies nutrition and oxygen. People having bladder dysfunction such as detrusor instability and/or impaired voiding ability increase in number with age[1–3] Clinically, atherosclerotic vascular disease, iatrogenic ligation of the hypogastric artery, pelvic thrombophrebitis, and ligation of the internal iliac artery at renal transplant are causes of bladder ischemia described in the literature[4–7].

Overdistention secondary to outflow obstruction also induces bladder ischemia[8–10] Dunn showed with the technetium-99m technique that the blood flow was significantly decreased by bladder wall overdistention[11] Although the effect of overdistention on the bladder blood flow has been studied, few reports have been made on the changes in function of the bladder following *in vivo* ischemia. In this study the effects of ischemia created by ligation of bilateral internal iliac arteries on the rat bladder function were studied by *in vivo* infusion cystometry and *in vitro* whole bladder study.

MATERIALS AND METHODS

Operation Procedure

Twelve male Sprague-Dawley rats (mean body weight 359.2 g, Chubu Kagaku Inc. Nagoya, Japan) were anesthetized with sodium pentobarbital (50 mg/kg). Through a lower midline incision the bilateral internal-iliac arteries (first branch of the common iliac artery) were exposed by retracting the seminal vesicle and the testicular vessels and were ligated with 5-0 silk. Six rats were subjected to ischemia for 7 days and the rest for 14 days. Sham surgery was done in the same way without ligating the arteries in 7 age- and weight-matched rats. After operation micturition was achieved by natural voiding without any assistance.

In Vivo Cystometry

Under subcutaneous urethane anesthesia (1.2 g/kg), the bladder was exposed gently by retracting both lobes of the prostate via suprapubic longitudinal incision. A double lumen catheter was intubated suprapubically through a small hole at the dome and fixed in the bladder with 4-0 silk. Ureters were not ligated. An outer catheter (outside diameter; 1.2 mm) was connected to a pressure transducer. Intravesical pressure was continuously recorded on a Rectigraph 8S (Model 180-4, San-ei Co., Tokyo, Japan). An inner catheter (outside diameter; 0.61

mm) was connected to an infusion pump (STC-521 Termo Co. Tokyo, Japan) and saline was infused. To eliminate the effect of infusion pressure on the intravesical pressure, the inner catheter was 2 mm longer than the outer one.

After the bladder and the prostate were returned to the normal position, cystometry was done at an infusion rate of 0.05 ml/min. The following parameters of cystometrogram were determined; maximum voiding pressure, capacity, residual urene volume, pressure at which micturition was initiated, voided volume, and voiding efficacy. Voiding efficacy was defined as voided volume/capacity×100.

***In Vitro* Whole Bladder Study**

After *in vivo* cystometry, both ureters and the urethra were ligated with 4-0 silk suture. The bladder was carefully dissected and put 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, $CaCl_2$ 2.5 mM, $NaHCO_3$ 25 mM, and glucose 11 mM). Saline (0.25 ml) was instilled into the bladder and the bladder was incubated for 30 min with a mixture of 95% O_2 and 5% CO_2.

Following incubation, pressure increases in response to field stimulation with frequencies ranging from 2 to 60 Hz, low and high dose of bethanechol (7.4 μM, 600 μM), maximum doses of ATP (2 mM), and high concentration of KCl (124 mM) were recorded. Field stimulation was applied through platinum electrodes placed on both sides of the bladder. Transmural nerve stimulation was performed by a stimulator DPS-160B (Dia Medical System Co., Tokyo, Japan) delivering biphasic square wave pulses of 50 volts, 0.5 ms duration. The interval of stimulations was 2 minutes. In a preliminary study, it was confirmed that over 90% of contractile response to field stimulation was blocked by 10^{-6} M tetrodotoxin. A high KCl solution was prepared by replacing NaCl with an equimolar amount of KCl.

Finally, a volume-pressure study was performed as follows; after intravesical saline was evacuated completely, the bladder was incubated for 30 min. Subsequently intravesical saline instillation was started and continued with an infusion rate at 0.05 ml/min. Field stimulation with 30 Hz was applied to the bladder and repeated with a 2 minutes interval. Passive pressure was determined as the intravesical pressure just before the bladder was stimulated. Active pressure was calculated as the maximal intravesical pressure provoked by field stimulation minus passive pressure.

Bethanechol and ATP were purchased from Sigma Co. Since bladder weight was different between sham-operated control and ischemic bladders, in the *in vitro* whole bladder study the pressure increases to various stimulations were normalized by tissue weight (cmH_2O/100 mg tissue).

Data are presented as mean±SEM. Statistical comparisons between ischemic and sham-operated control bladders were made with an unpaired Student's t-test. A level of $p<0.05$ was accepted as statistically significant.

RESULTS

Bladder weight significantly increased 14 days after ischemia (Table 1). Analysis of parameters of *in vivo* cystometrogram showed that voiding pressure significantly decreased, and that capacity and residual urine volume significantly increased, which in turn resulted in significant decrease in voiding efficacy (Table 1). Pressure at which micturition was initiated, and voided volume were unchanged. Representative tracings of cystometrogram of the sham-operated control and ischemic bladders were shown in Figure 1.

In vitro whole bladder study demonstrated that pressure changes in response to low frequencies of field stimulation significantly decreased both 7 days and 14 days after ischemic surgery (Fig. 2). The responses to the low and high doses of bethanechol were significantly suppressed by ischemia on the 14th day (Fig. 3). The response to ATP significantly decreased on the 7th and 14th day (Fig. 4). The response to KCl was significantly reduced on the 14th day (Fig. 5).

Table 1. The effect of *in vivo* ischemia on the cystometric parameters.

	Sham-operated	Ischemia	
	Control	7 days	14 days
Number	7	6	6
Bladder weight (mg)	110.5 ±8.8	122.2 ±5.0	*147.1 ±15.0
Voiding pressure (cmH_2O)	14.7 ±0.83	*6.3 ±1.07	*9.8 ± 1.71
Capacity (ml)	0.26±0.04	0.34±0.08	*0.50± 0.08
Residual urine volume (ml)	0.001±0.001	*0.11±0.04	*0.21± 0.04
Pressure at which micturition was initiated (cmH_2O)	3.0 ±0.32	4.8 ±1.03	3.8 ± 0.60
Voided urine volume (ml)	0.26±0.04	0.22±0.07	0.30± 0.06
Voiding efficacy (%)	99.5 ±0.5	*74.6 ±7.2	*58.7 ± 6.0

Each value is mean±SEM. * Significant difference from the value of sham-operated control bladders, $p<0.05$.

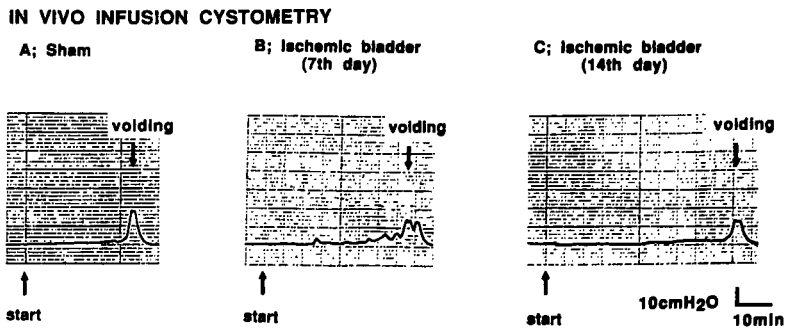

Fig. 1. Representative cystometrograms of the sham-operated control bladder (A), and the ischemic (B ; 7 days, C ; 14 days) bladders.

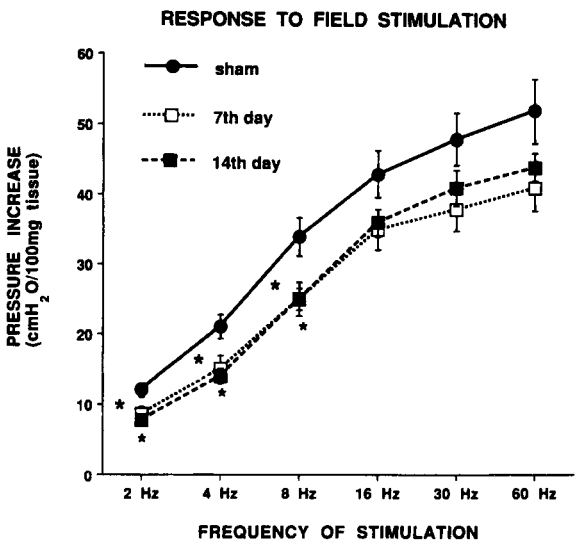

Fig. 2. The effect of ischemia on pressure increase of the *in vitro* whole bladder in response to field stimulation. Each point is the mean±SEM of 7 sham or 6 ischemic bladders ; significant difference from the value of sham-operated control bladeers, $p<0.05$.

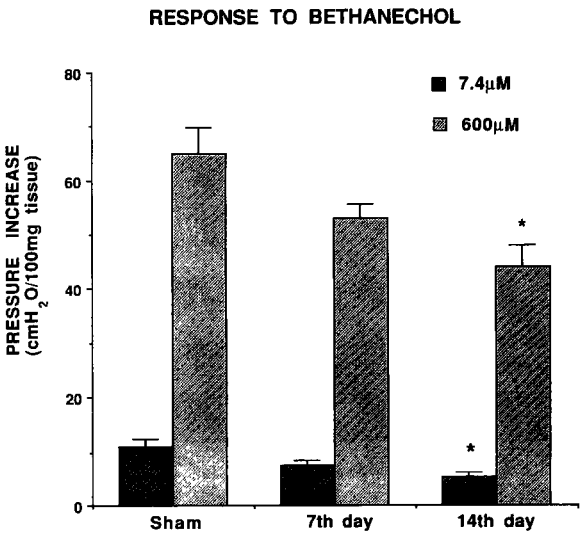

Fig. 3. The effect of ischemia on the pressure increase of *in vitro* whole bladder in response to low and high doses of bethanechol. Each bar is the mean± SEM of 7 sharm or 6 ischemic bladders ; * significant difference from the value of sham-operated control bladders, $p<0.05$.

Although passive volume-pressure curve slightly shifted to the left (decrecse in compliance) on the 7th day, it shifted to the right (increase in compliance) on the 14th day (Fig. 6). The peak response in an active volume-pressure curve decreased after ischemia and shifted to the right 14 days after surgery (Fig. 7). The peak response was encountered at 0.2 ml of the bladder capacity in the sham-operated control and in the 7th-day ischemic bladders, respectively whereas this was observed at 0.4 ml in the 14th-day ischemic bladders.

DISCUSSION

Atherosclerosis is a common pathophysiological change seen in elderly people. Decreased blood supply to the bladder would be expected to deteriorate its function. Ischemia of the urinary bladder is associated with a variety of detrimental conditions such as increased susceptibility to infections, impaired emptying power, and necrosis of the bladder[4–7,10,12] Bladder dysfunction following outflow obstruction might be closely related to ischemia with overdistention[8–10]

The contraction of the detrusor muscle is initiated and maintained by excitation of the parasympathetic nerves. Acetylcholine excreted from the nerve terminals stimulates muscarinic cholinergic receptors. In several mammals some part of the contraction induced by nerve stimulation shows resistance to atropine[13] The primary candidate of the transmitters responsible for the atropine resistant component of the response is ATP (purinergic transmitter)[14,15] Receptor activation opens receptor-operated calcium channels. Consequently, the smooth muscle contraction is induced by increase in intracellular free calcium concentration[16,17] On the smooth muscle cell membrane there is another kind of calcium channel which is independent of receptor function. That is voltage-dependent calcium channels whose function is primarily

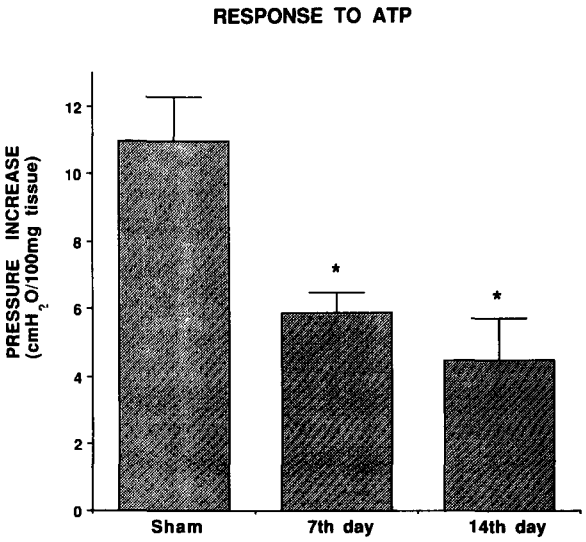

Fig. 4. The effect of ischemia on the pressure increase of *in vitro* whole bladder in response to ATP. Each bar is the mean±SEM of 7 sham or 6 ischemic bladders ; * significant difference from the sham-operated control bladders, $p<0.05$.

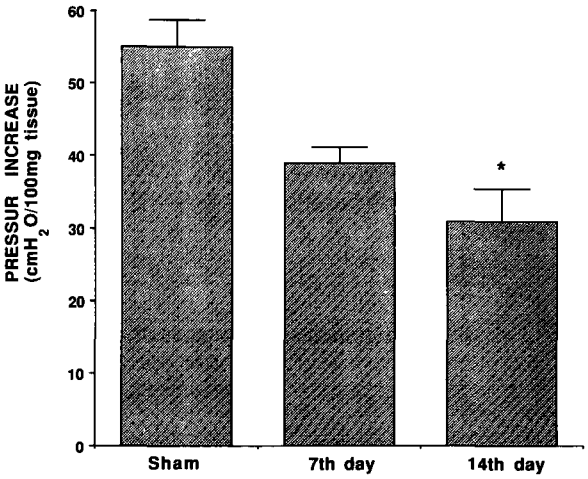

Fig. 5. The effect of ischemia on the pressure increase of *in vitro* whole bladder in response to KCl. Each bar is the mean±SEM of 7 sham or 6 ischemic bladders ; * significant difference from the value of sham-operated control bladders, $p<0.05$.

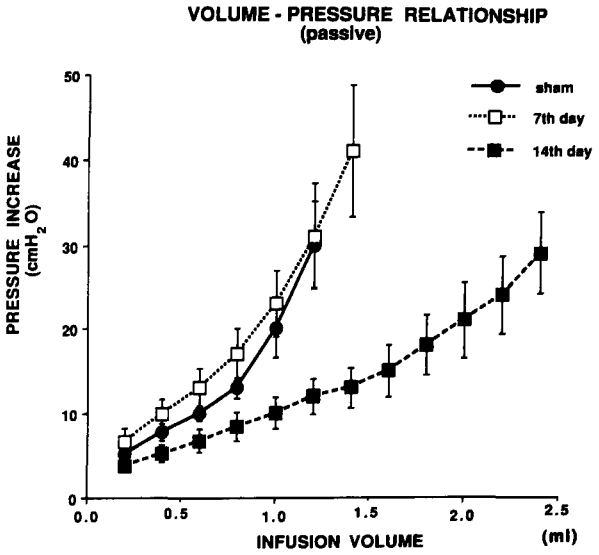

Fig. 6. The effect of ischemia on the passive pressure increase in relation to intravesical volume. Each point is the mean±SEM of 7 sham or 6 ischemic individual bladders.

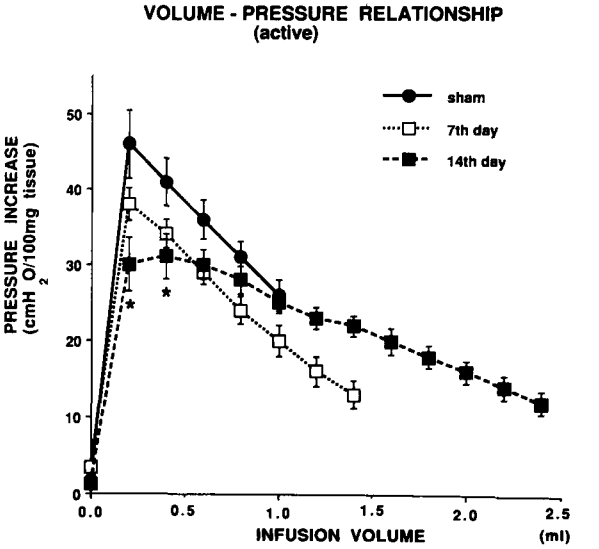

Fig. 7. The effect of ischemia on the active pressure increase in response to 30 Hz field stimulation in relation to intravesical volume. Each point is the mean±SEM of 7 sham or 6 ischemic individual bladders ; * significant difference from the value of sham-operated control bladders, $p<0.05$.

dependent on the potential of the muscle cell membrane. The smooth muscle can be depolarized by a high concentration of KCl.

In the current study ischemic bladder showed significant reduction of voiding pressure and significant increase in residual urine volume *in vivo*. These findings suggest that ischemia impaired bladder voiding ability. In an *in vitro* study, we evaluated the effects of ischemia on the detrusor contractility to different kinds of stimulation. Electrical field stimulation activated the intramural nerve which was sensitive to tetrodotoxine. Bethanechol (non-hydrolytic cholinergic agent) stimulated directly muscarinic cholinergic receptors. ATP (adenosine 5'-triphosphate) contracted the rat detrusor through purinergic receptors. High KCl depolarized the smooth muscle cell membrane. The fact that *in vitro* responses to these stimuli were all impaired after ischemic surgery suggested that some pathological alteration of detrusor, i.e., myogenic degeneration, took place. These findings are consistent with those obtained in a rabbit study[7,18–20] Saito (one of us) had determined

changes in blood flow by laser Doppler flowmetry. His experiments showed that under zero capacity blood flow decreased to 32% of control immeriate after ligation of the bilateral internal iliac arteries, then returned to 44% on the 7th day. Finally on the 14th day it recovered to almost the control level (unpublished data). This significant decrease in blood supply can cause the degeneration of the detrusor smooth muscle.

In a passive volume-pressure study bladder compliance once decreased 7 days after surgery but increased 14 days later. The former may be related to the extensive edema present in the mucosal layer (unpublished data), and the latter an increased bladder capacity which was demonstrated in the *in vivo* study. Gill et al. observed a similar fall of compliance in their acute rabbit experiment of ligating the vesical arteries and postulated that edema and congestion in the bladder wall was responsible for the change[7]

In general, the strength of muscle contraction depends on its length[21] However, if the muscle is too elongated, the tension will be reduced. An active pressure-volume study was performed to determine the strength of detrusor contractility at each volume in response to field stimulation. We found that decrease in the peak response in ischemic bladder was consistent with weakened contractility to field stimulation *in vitro* and lowered voiding pressure *in vivo*. The active pressure-volume curve of the control was the same in shape with that on the 7th day but was different from that on the 14th day with less steep decline in the pressure increase at the larger volume, which may reflect the enhanced bladder compliance 2 weeks later.

In conclusion, ischemia of the bladder weakened detrusor contractility to empty urine *in vivo*. Decreased contractility of the whole bladder *in vitro* in response to several stimuli suggested that myogenic degeneration of the detrusor was the cause of the deteriorated bladder function.

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和文抄録

ラットの膀胱内圧測定および摘出膀胱収縮力に対する虚血の影響

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動脈硬化に伴う虚血は高齢者におけるさまざまな器官の機能障害の原因と考えられる．また，高齢者の排尿障害の一因として膀胱の血流障害が考えられている．そこで，ラットの両側内腸骨動脈を結紮し虚血ラット膀胱を作成し，この虚血膀胱における urethane 麻酔下の膀胱内圧測定および摘出膀胱の電気刺激，bethanechol，ATP，KCl に対する収縮力を検討した．ついで一定速度で生理食塩水を膀胱内に注入しながら電気刺激を繰り返し volume-pressure study を実施した．膀胱の重量は虚血により有意に増大した．膀胱内圧曲線を分析した結果，膀胱容量，残尿量は増大し，排尿筋収縮圧は減少した．この結果，虚血に伴い排尿効率（排尿量/膀胱容量）は有意に減少した．摘出膀胱における排尿収縮力は，すべての刺激に対して虚血後に低下した．volume-pressure study の結果から *in vitro* の膀胱 compliance は 7 日後減少し14日後は逆に増大した．以上の結果より，ラット膀胱では虚血により膀胱収縮力が低下し，排尿効率は悪化した．この原因は摘出膀胱の収縮力がすべての刺激に対して低下したことにより，膀胱平滑筋自体の虚血に伴う変化によると考えられた．

（泌尿紀要 **42**：117-122，1996）

Editorial Comment

Voiding dysfunction in the patients with benign prostatic hypertrophy (BPH) has long been attributed to possible bladder outlet obstruction (BOO). With the advent of urodynamics particularly with clinical application of pressure flow studies, voiding dysfunction primarily attributable to detrusor weakness (DW) has emerged. In BPH it is relevant to differentiate BOO from DW because outcome of treatment modalities aimed at relieving BOO is satisfactory in BOO, but not so much in DW. The clinical relevance notwithstanding we have little knowledge as to the etiology of DW. The authors are to be commended in creating an animal model of DW induced by ischemia from ligation of hypogastric artery. From meticulous experimental works in this model they concluded that the detrusor muscle degenerated to the extent that its power was impaired.

In clinical practice, the risk of damage to the upper tract is less in DW as compared to BOO. It would be interesting to see if this model poses less threat to the upper tract as opposed to the BOO model, which obviously could not be answered in this short-term experiment.

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