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<td>Author(s)</td>
<td>YOKOI, Keisuke; OHMURA, Masaharu; KONDO, Atsuo; MIYAKE, Koji; SAITO, Masahiko</td>
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Kyoto University
EFFECTS OF IN VIVO ISCHEMIA ON THE INFUSION CYSTOMETRY AND IN VITRO WHOLE BLADDER CONTRACTILITY OF THE RAT

Keisuke Yokoi, Masaharu Ohmura, Atsuo Kondo, Koji Miyake and Masahiko Saito
From the Department of Urology, Nagoya University School of Medicine

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.

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. Clinically, atherosclerotic vascular disease, iatrogenic ligation of the hypogastric artery, pelvic thrombophlebitis, and ligation of the internal iliac artery at renal transplant are causes of bladder ischemia described in the literature.

Overdistention secondary to outflow obstruction also induces bladder ischemia. Dunn showed with the technetium-99m technique that the blood flow was significantly decreased by bladder wall overdistention. 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 suprapublically 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 urine 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, MgSO4 1.2 mM, KH2PO4 1.2 mM, CaCl2 2.5 mM, NaHCO3 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% O2 and 5% CO2.

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 waves 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 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 (cmH2O/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.**

<table>
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<th>Sham-operated</th>
<th>Ischemia</th>
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<tr>
<td></td>
<td>Control</td>
<td>7 days</td>
</tr>
<tr>
<td>Number</td>
<td>7</td>
<td>6</td>
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<tr>
<td>Bladder weight (mg)</td>
<td>110.5±8.8</td>
<td>122.2±5.0</td>
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<tr>
<td>Voiding pressure (cmH2O)</td>
<td>14.7±0.83</td>
<td>*6.3±1.07</td>
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<tr>
<td>Capacity (ml)</td>
<td>0.26±0.04</td>
<td>0.34±0.08</td>
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<tr>
<td>Residual urine volume (ml)</td>
<td>0.001±0.001</td>
<td>*0.11±0.04</td>
</tr>
<tr>
<td>Pressure at which micturition was initiated (cmH2O)</td>
<td>3.0±0.32</td>
<td>4.8±1.03</td>
</tr>
<tr>
<td>Voided urine volume (ml)</td>
<td>0.26±0.04</td>
<td>0.22±0.07</td>
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<tr>
<td>Voiding efficacy (%)</td>
<td>99.5±0.5</td>
<td>*74.6±7.2</td>
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Each value is mean±SEM. * Significant difference from the value of sham-operated control bladders, p<0.05.
IN VIVO INFUSION CYSTOMETRY

A; Sham
B; Ischemic bladder (7th day)
C; Ischemic bladder (14th day)

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

RESPONSE TO FIELD STIMULATION

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<tr>
<td>2 Hz</td>
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<tr>
<td>sham</td>
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<tr>
<td>7th day</td>
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<td>14th day</td>
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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 bladders, p<0.05.

Although passive volume-pressure curve slightly shifted to the left (decrease 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. Bladder dysfunction following outflow obstruction might be closely related to ischemia with overdistention.

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. The primary candidate of the transmitters responsible for the atropine resistant component of the response is ATP (purinergic transmitter). Receptor activation opens receptor-operated calcium channels. Consequently, the smooth muscle contraction is induced by increase in intracellular free calcium concentration. 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
RESPONSE TO ATP

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.

RESPONSE TO KCl

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.

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.

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.

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. 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 immediately 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.

In general, the strength of muscle contraction depends on its length. 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 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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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.

Tomohiko Koyanagi
From the Department of Urology, Faculty of Medicine, Hokkaido University