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Effect of ischemia and partial outflow obstruction on rat bladder function

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Abstract We investigated the effects of ischemia induced by ligation of the bilateral internal iliac arteries following partial outlet obstruction on changes in detrusor function in rat. Rats were divided into three groups: sham-operated control rats, rats with partial outlet obstruction, and rats with obstruction + ischemia. Bladder function was studied by the in vitro organ bath technique 7 days after surgery. The weight of the bladder was significantly increased in both the obstruction and obstruction + ischemia groups. The obstruction + ischemia group exhibited a greater increase in weight. The passive length-tension relationship of detrusor muscle strips showed that tissue elasticity was decreased and the active length-tension relationship demonstrated that the peak response was observed at a shorter tissue length in the obstruction + ischemia group compared with the other two groups. There was no difference in the passive and active length-tension relationships between the control group and the obstruction group. The contractile response to various kinds of stimulation (field stimulation, bethanechol, ATP, and KCl) increased in the obstruction group and decreased in the obstruction + ischemia group. These findings suggest that partial outflow obstruction alone increased bladder contractility in response to stimuli. However, ischemia reduced the contractility and elasticity of the bladder wall.

Key words Outflow obstruction · Rat bladder · Ischemia · Detrusor function · Atherosclerosis · Benign prostatic hyperplasia

Introduction

Bladder outflow obstruction due to benign prostatic hyperplasia (BPH) causes either irritating symptoms, such as urgency or urge incontinence, due to detrusor hyperactivity, or obstructive symptoms, such as a weak stream or incomplete evacuation, caused by increased urethral resistance and/or impaired detrusor contractility. Animal experiments have suggested that mild short-term partial outflow obstruction increases detrusor contractility [6, 7, 15], but that severe, long-term obstruction impairs detrusor contractility [7, 10, 16, 17]. These experimental findings may not be valid in all clinical situations, in which unknown etilogies may be involved [5,20]

An adequate blood flow supplies nutrition and oxygen, maintaining normal bladder function. Thus, ischemia related to atherosclerosis may affect bladder function. In a previous study, we found that ischemia induced by ligation of the internal iliac arteries reduced detrusor contractility in rat [13]. In the present study, we examined the effects of ischemia and outflow obstruction on functional changes in the rat bladder.

Materials and methods

We divided 42 male Sprague-Dawley rats (Chubu Kagaku, Aichi, Japan) weighing 300–350 g into three groups: partial outflow obstruction (obstruction group), partial outflow obstruction and ligation of internal iliac arteries (obstruction + ischemia group), and sham operation (control).

Operative procedure

Rats were anesthetized with sodium pentobarbital (50 mg/kg). Surgery was performed using sterile technique. After a midline suprapubic incision was made, the bilateral lobes of the prostate were retracted to expose the bladder neck and urethra without causing damage to the bladder. The space surrounded by the ureter, the urethra, and the vas deferens was dissected and a catheter (OD 1.67 mm) was placed on the urethra. A 2-0 silk suture was passed behind the urethra, the urethra was ligated, and the catheter was removed. The catheter was used to ensure precise and reproducible degrees of urethral obstruction. In the obstruction + ischemia group, the seminal vesicle and spermatic vessels were gently retracted to expose the internal iliac arteries. The bilateral internal

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iliac arteries were ligated with 5-0 silk sutures and cut. Specimens were prepared for in vitro experiments 7 days after surgery. Previous studies have shown that the effects of obstruction and ischemia on detrusor function are apparent 7 days after surgery [13,15]

In vitro study of muscle strips

After the bladder weights were recorded, two longitudinal strips (8 × 1.5 mm) were created from the body of the bladder. The muscle strips were suspended in individual organ baths containing 10 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) at 37°C with a mixture 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-8k (San-ei Co., Tokyo, Japan).

Length-tension relationships

The active and passive length-tension relationships of the muscle strips were examined according to the method described by Andersson et al [1]. Briefly, muscle strips were first stretched with 1 g resting tension in normal Krebs' solution and then allowed to relax for 30 min. The tissue samples were then stimulated with high-dose potassium Krebs' solution (KCl 124 mM). The increase in tension was canceled out with a manipulator that shortened the tissue length. Muscle strips were bathed with calcium-free Krebs' solution for 15 min. The procedure was performed 3 times to obtain complete relaxation of the muscle specimens. Muscle strips were then stretched 2 mm with the manipulator and the decay in tension was observed for 10–15 min (stress relaxation phenomenon). The final tension was defined as the passive tension. The incubation medium was replaced with KCl Krebs' solution to contract the muscle, and the maximal increase in tension was defined as the active tension. The medium was then replaced with calcium-free solution, and tissue strips were again stretched 2 mm. These steps were performed 12 times for the control and obstruction groups, and 6 times for the obstruction + ischemia group.

Contractile response to stimuli

Muscle strips were equilibrated for 30 min. The resting tension was adjusted to around 1 g at the end of the incubation period. We examined changes in tension in response to field stimulation (2–60 Hz), bethanechol (0.8–600 μM), and maximal doses of ATP (2 mM) and KCl (124 mM). For field stimulation, platinum electrodes were placed on both sides of the muscle strip in the organ bath. Transmural nerve stimulation was applied with a field stimulator (DPS-160, Dia-Medical System, Tokyo, Japan) that delivered biphasic square-wave pulses of 50V and 0.5ms in duration at 2-min intervals.

Drugs

Bethanechol and adenosine 5'-triphosphate (ATP) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). A high-dose potassium solution was prepared by replacing NaCl with an equimolar amount of KCl in Krebs' solution

Statistical analysis

The active tension increase in the length-tension relationship and the contractile response to stimuli were normalized by the tissue weight (g/100 mg tissue). The passive length-tension relationship is expressed as the absolute value in grams. Data were analyzed by Fisher's protected least significant difference test. A P level < 0.05 was accepted as statistically significant.

Results

The bladder weight was significantly increased in both the obstruction and the obstruction + ischemia groups compared with the control group. The increase was more pronounced in the obstruction + ischemia group (Fig. 1). The passive length-tension relationship indicated that obstruction + ischemia reduced tissue elasticity (Fig. 2). The active length-tension relationship demonstrated that the peak response occurred at a shorter tissue length in the obstruction + ischemia group than the other two groups (Fig. 3). Obstruction increased the response to the high frequencies of field stimulation, but obstruction + ischemia significantly decreased the response at all frequencies (Fig. 4). The

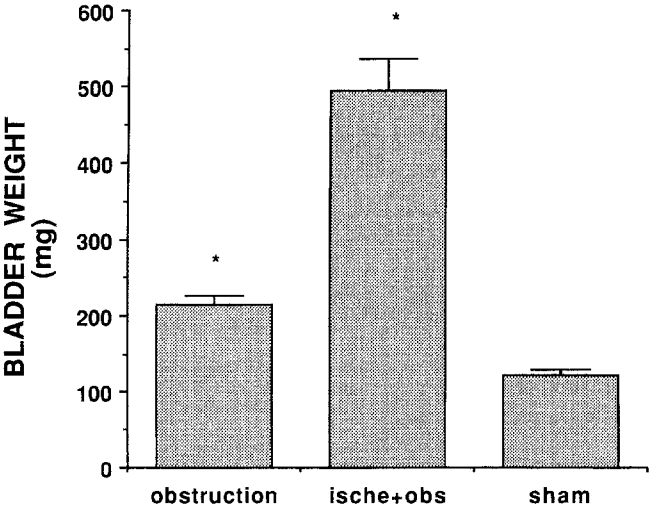

Fig. 1 Effects of obstruction and obstruction + ischemia on the bladder weight. *Bars* represent the means ± SEM of 12 individual observations. * Significant difference from the sham-operated control, $P < 0.05$

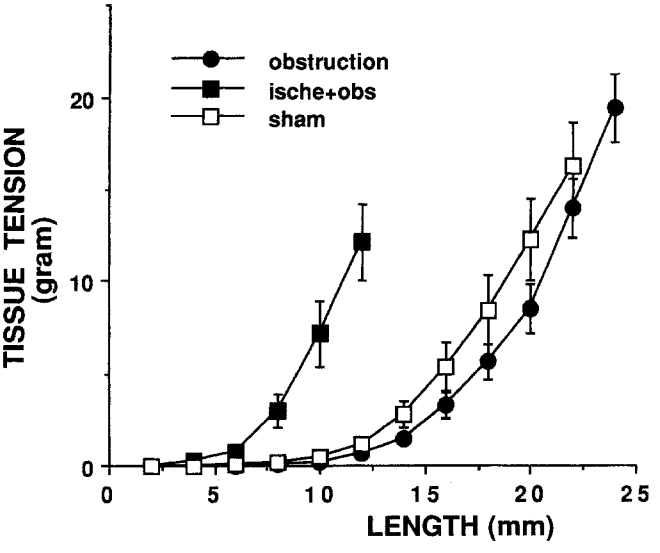

Fig. 2 Passive length-tension relationships in the three groups. *Each point* is the mean ± SEM of six duplicate observations

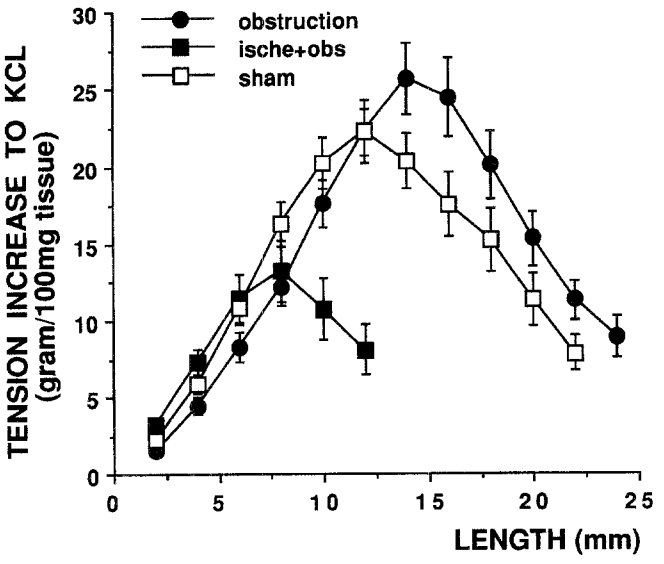

Fig. 3 Active length-tension relationships in the three groups. *Each point* is the mean ± SEM of six duplicate observations

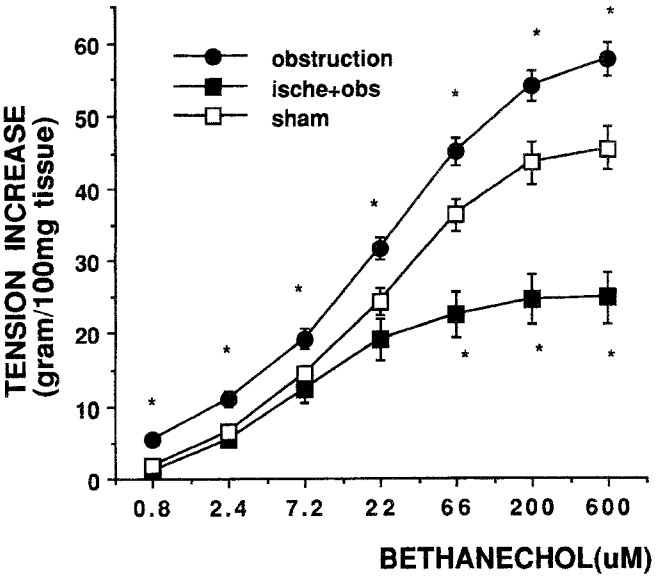

Fig. 5 Effects of obstruction and obstruction + ischemia on the response to bethanechol. *Each point* is the mean ± SEM of six duplicate observations. * Significant difference from the sham-operated control, $P < 0.05$

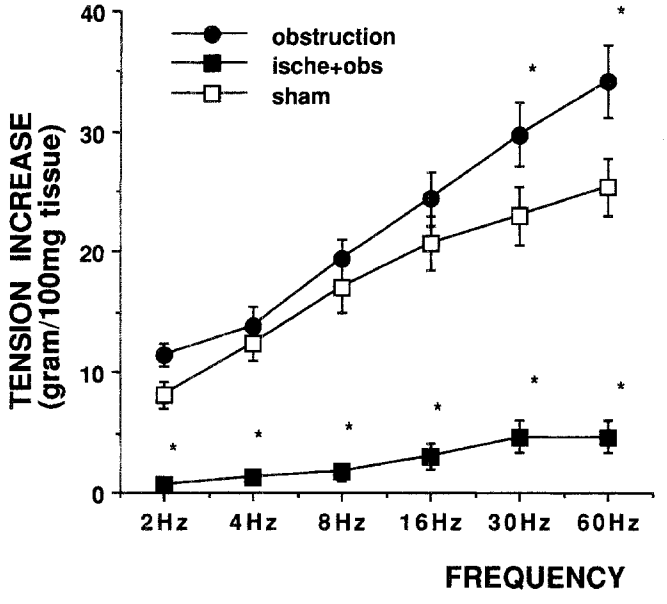

Fig. 4 Effects of obstruction and obstruction + ischemia on the response to field stimulation. *Each point* is the mean ± SEM of six duplicate observations. * Significant difference from the sham-operated control, $P < 0.05$

response to bethanechol was increased in the obstruction group but was decreased in the obstruction + ischemia group (Fig. 5). Although the response to ATP increased in the obstruction group, there was no difference between the sham-operated control group and the obstruction + ischemia group (Fig. 6). The response to KCl was significantly decreased by obstruction + ischemia (Fig. 7).

Discussion

The proper function of smooth muscle requires the normal perfusion of the tissue with blood and the supply of oxygen and nutrients. Atherosclerosis associated with aging reduces blood flow and leads to deterioration of the function of various organs. Tissue ischemia can affect detrusor function.

In a previous study, the partial ligation of the urethra for up to 28 days increased the nocturnal frequency of micturition in vivo and enhanced detrusor contractility in vitro in rat [15]. These changes were attributed to the compensatory response of the detrusor to increased urethral resistance. In the present study, obstruction increased the contractile response to stimuli, which is consistent with the results of a previous study [13]. This means that the degree of outflow obstruction is constantly mild when we used the 1.67-mm-outside-diameter (OD) catheter at surgery. However, ischemia impaired detrusor contractility in association with a marked increase in bladder weight and reduced tissue elasticity. These findings suggest that the bladder requires adequate blood circulation to compensate for an increased outflow resistance.

In general, blood flow to the bladder decreases as the bladder capacity increases [3,11,12,18]. Therefore, overdistension secondary to outflow obstruction induces chronic bladder ischemia. Dunn found that blood flow was significantly decreased by overdistension of the bladder wall in a study using technetium-99m [2]. Lin et al. [9] observed an 80% decrease in blood flow in obstructed rabbit bladders. A decrease in blood flow following outflow obstruction is reversible after the ob-

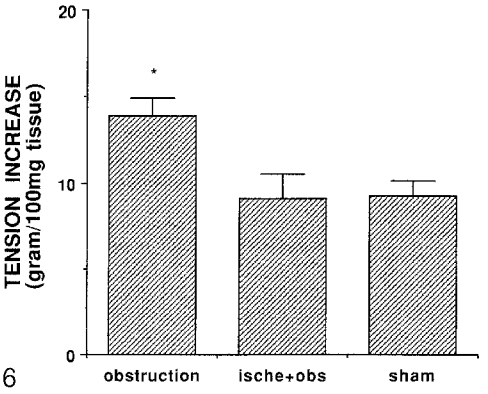

Fig. 6 Effects of obstruction and obstruction + ischemia on the response to ATP. *Each point* is the mean ± SEM of six duplicate observations. * Significant difference from the sham-operated control, $P < 0.05$

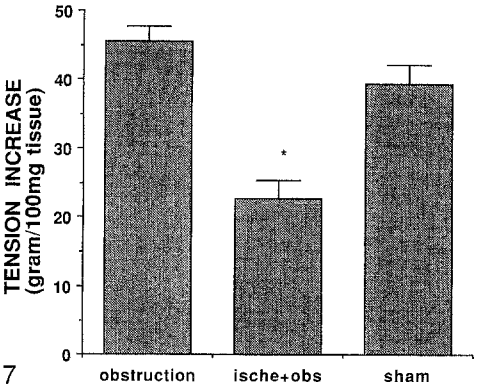

Fig. 7 Effects of obstruction and obstruction + ischemia on the response to KCl. *Each point* is the mean ± SEM of six duplicate observations. * Significant difference from the sham-operated control, $P < 0.05$

struction is released [9]. Ischemic changes in the bladder wall are believed to be important contributors to functional deterioration. We have found that blood flow to the bladder is unchanged following mild obstruction (OD) of the catheter 1.67 mm), but decrease significantly after severer obstruction (OD of the catheter 1.10 mm) in association with impaired detrusor contractility (unpublished observations). Thus, ischemia of the bladder significantly impairs detrusor contractility in animals with outflow obstruction.

Four kinds of stimulation applied in the present study were chosen as representative stimulation for each stage of detrusor contraction. Field stimulation induces the detrusor contraction through intramural nerve stimulation. Bethanechol and ATP stimulate muscarinic-cholinergic and purinergic receptors, respectively. A high concentration of KCl contracts detrusor via depolarizing of smooth muscle cell membrane, which is independent of receptor function. Unpublished studies from our laboratory have demonstrated that immediately after ligation of both sides of the internal iliac arteries blood flow to the bladder decreases to 30% of presurgical level. Histologically at the 7th day the bladder wall had inflammatory changes especially in the mucosa and submucosal layer and degeneration of smooth muscle. Severe inflammation induced by ischemia could have contributed to an increase in bladder weight in the obstruction + ischemia group. Since contractile response to field stimulation, bethanechol, and KCl were decreased by ischemia, smooth muscle degeneration was thought to be a primary factor which resulted in a decreased response to stimuli in the obstruction + ischemia group.

The decrease in the response to field stimulation was greater than the decrease in the responses to direct receptor stimulation or direct smooth muscle depolarization in the obstruction + ischemia group in the present study. This finding is similar to the changes in detrusor function observed in rat bladders with severe outflow obstruction [16]. The difference between the response to field stimulation and the response to pharmacologic stimuli suggests that the intramural nerve in the detrusor is more sensitive to ischemia than the smooth muscle. Gosling et al. [4] observed a decrease in the number of autonomic innervations in the detrusor of human bladders with outflow obstruction. Levin et al. [8] found that choline-acetyltransferase activity was decreased in rabbit bladders with outflow obstruction in which detrusor contractility was significantly impaired. It should be noted that contraction of detrusor subjected to ischemia + obstruction in response to ATP did not decrease when compared to control. Some literature reported that the outflow-obstructed bladder or neurogenic bladder demonstrates an increased response to ATP, which is thought to contribute to increased atropine resistance observed in pathological bladders [14, 19]. Therefore, the well-preserved contractility to ATP might be attributed to the relatively increased contractility to ATP induced by obstruction and ischemia.

In summary, partial outflow obstruction alone increased bladder contractility in response to various stimuli in rats, while ischemia reduced contractility and the elasticity of the bladder wall. These findings suggest that tissue ischemia may inhibit the compensatory ability of the detrusor in response to increased urethral resistance and that ischemic changes may result in a noncompliant noncontractile bladder.

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