

EFFECTS OF ISCHEMIA ON THE FUNCTION OF THE ISOLATED RAT DETRUSOR MUSCLE

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Ischemia of vital organs causes various degrees of impairment. We studied the *in vitro* effects of ischemia on the function of the rat bladder. Ischemia was induced by ligation of the bilateral (bilateral ischemia) or right (unilateral ischemia) internal iliac arteries.

Bladder weight increased significantly following 1 week of bilateral and unilateral ischemia. Passive tension was significantly higher in ischemic bladders than in control bladders at an increase in length between 6 and 12 mm. In both control and ischemic bladders, active tension was highest at a 16-mm increase in length. Bladders subjected to unilateral or bilateral ischemia for 1 or 2 weeks demonstrated impaired contractile responses to field stimulation, bethanechol, ATP and KCl. There were no differences in contractile strength between muscle specimens obtained from the ipsilateral or contralateral sides of unilateral ischemic bladders. Our findings showed that unilateral and bilateral ischemia inhibited the *in vitro* contractile strength of the detrusor muscle in response to intramural and pharmacologic stimulation.

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Key words: Rat bladder function, Ischemia

INTRODUCTION

The proper functioning of any smooth muscle system requires the normal perfusion of the tissue with blood, oxygen, and nutrients¹⁾ Ischemia of the bladder may deteriorate detrusor function e.g. bladder instability or impaired detrusor contractility. Ischemia inhibits tissue metabolism by depriving the tissue of oxygen and nutrients and by inducing accumulation of waste products. Experimentally acute ischemia (for 1 hour) reduced the detrusor contractility in association with a decrease in the intracellular content of ATP²⁾. Short-term ischemia induced by ligation of the vesical arteries was found to reduce detrusor contractility and to impair bladder compliance in rabbits³⁾ We investigated the effects of ischemia on bladder function in a rat model.

MATERIALS AND METHODS

Operative Procedure: Because ligation the vesical artery without causing damage to the nerves or ureters is technically difficult, we induced ischemia by ligating the internal iliac arteries. We confirmed that the first branch of the common iliac artery (the internal iliac artery) supplied blood to part of the seminal vesicle, to part of the prostate, and to the whole bladder. Preliminary experiments showed that there were no differences in the *in vitro* contractile response of muscle strips to various kinds of stimulation in the effects of ischemia induced by ligation of the common iliac arteries and ligation of the internal iliac arteries (data not shown).

Male Sprague-Dawley rats of approximately 350 g

(Chubu Kagaku Inc. Nagoya, Japan) were anesthetized with sodium pentobarbital (50 mg/kg). A midline suprapubic incision was made with rats in the supine position. After the seminal vesicles and testicular vessels were gently retracted, the internal iliac artery was identified, ligated with 5-0 silk sutures, and cut. Bilateral ischemia was induced by ligating the internal iliac artery bilaterally (n=6). Unilateral ischemia was induced by ligating the right artery (n=6). The *in vitro* effects of ischemia were examined 1 and 2 weeks after surgery. The control group consisted of ages and sex-matched rats (n=6).

Preparation of Muscle Strips: Bladders were excised from ischemic and control rats anesthetized with pentobarbital (50 mg/kg) and placed in Krebs' solution. The surrounding fatty tissue was cleaned off, and bladders were weighed. Two longitudinal muscle strips with unstretched size of about 8×1.5 mm (mean weight: 27.6±1.8 mg) were obtained from the body of the bladder.

Length-tension Relationship: The active and passive length-tension relationships of the muscle strips were examined in the control group and the bilateral ischemia group at 1 week. Two longitudinal muscle strips (8×1.5 mm) were suspended in 20°C Krebs' solution. Tissue strips were mounted in an organ bath containing Krebs' solution at 37°C. One end of each strip was connected to a force displacement transducer (Model 45196 San-ei Co. Nagoya, Japan). Changes in tension were measured and recorded on a Rectigraph-8K (San-ei Co.). Length-tension studies were performed using the method described by Andersson

et al.⁴). Briefly, muscle strips were first stretched with 1 g of resting tension and then allowed to relax for 30 min. The tissue samples were then stimulated with a high-dose potassium Krebs' solution (KCl 124 mM). The increase in tension was cancelled out by using a manipulator that shortened the tissue length. Muscle strips were bathed with calcium-free Krebs' solution for 15 min. Steps 2 and 3 were repeated three times to obtain complete relaxation of the muscle specimen. Muscle strips were then stretched 2 mm with the manipulator and the decay in tension was observed for 10 to 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 increase in active tension. The medium was then replaced with calcium-free Krebs' solution, and tissue strips were again stretched 2 mm. Specimens were elongated in 2-mm steps for a total of 10 times.

In Vitro Muscle Strip Study: Each strip was suspended in an organ bath containing 10 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) aerated with 95% oxygen and 5% carbon dioxide. After the strip was equilibrated for 60 minutes with 1 g of resting tension, the effects of field stimulation, bethanechol, ATP, and KCl on tension were determined. 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, Japan) delivering biphasic square-wave pulses of 50 volts of 0.5-ms durations at 2-min intervals. Stimulation was applied at frequencies ranging from 2 to 60 Hz.

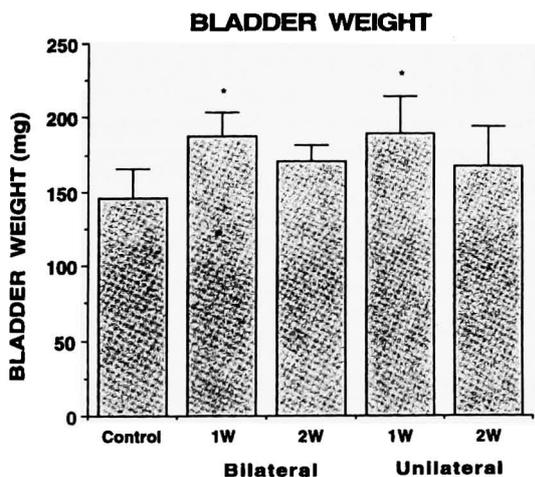


Fig. 1. Effect of bilateral and unilateral *in vivo* ischemia on bladder weight. Bars represent the mean \pm SEM of 6 individual observations. * Significant difference from the control bladder, $p < 0.05$

The dose-response effect of bethanechol on the bladder was investigated using doses ranging from 0.8 to 600 μ M. The maximal responses to ATP (2 mM) and KCl Krebs' solution (124 mM) were determined. KCl Krebs' solution was prepared by replacing whole NaCl in normal Krebs' solution with an equimolar amount of KCl.

Drugs: Bethanechol and ATP were purchased from Sigma Chemical Co. (Japan).

Statistical Analysis: The increase in passive tension was expressed as an absolute value in grams. The other contractile responses to stimulations were expressed in grams of tension/100 mg of tissue. Data were analyzed by the unpaired Student's t-test.

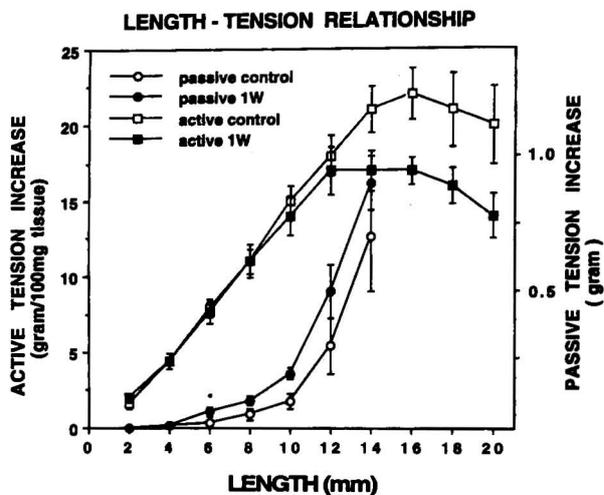


Fig. 2. Length-tension relationships in the control and bilateral ischemia groups at 1 week. To clearly depict significant difference in passive length-tension curve, data from 16 to 20 mm were not shown. Each point is the mean \pm SEM of 6 duplicate observations. * Significant difference from the control response, $p < 0.05$

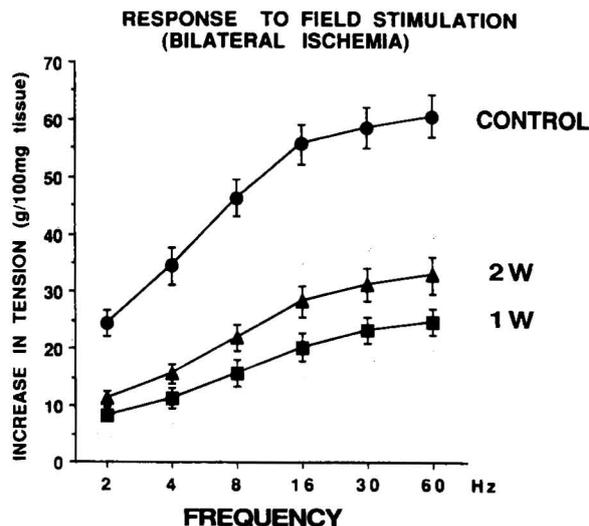


Fig. 3. Effect of bilateral ischemia on bladder responses to field stimulation. Each point is the mean \pm SEM of 6 duplicate observations. * Significant difference from the control response, $p < 0.05$

$p < 0.05$ was accepted as statistically significant.

RESULTS

The bladder weight increased significantly at 1 week in the bilateral and unilateral ischemia groups compared with the control group. There was no difference in weight among groups at 2 weeks (Fig. 1).

The increase in passive tension was significantly higher in ischemic bladders than in control bladders at an increase in length between 6 and 12 mm (Fig. 2). Maximal active tension was observed at an increase of 16 mm in both the control and ischemic bladders, and was significantly higher in control bladders. The bladders subjected to either unilateral or bilateral ischemia for 1 or 2 weeks demonstrated decreased responses to field stimu-

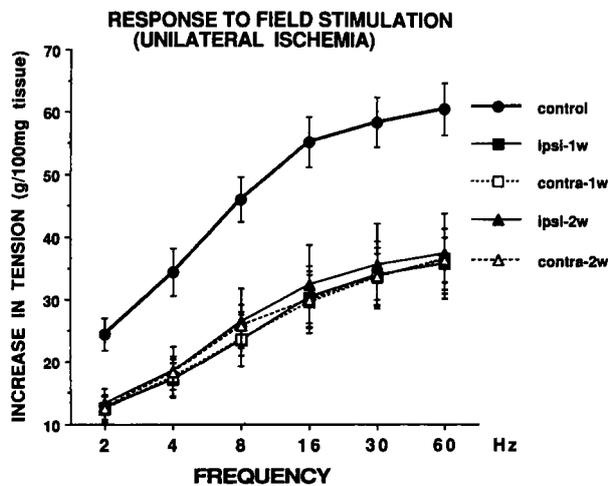


Fig. 4. Effect of unilateral ischemia on responses to field stimulation. Each point is the mean \pm SEM of 6 duplicate observations. * Significant difference from the control response, $p < 0.05$

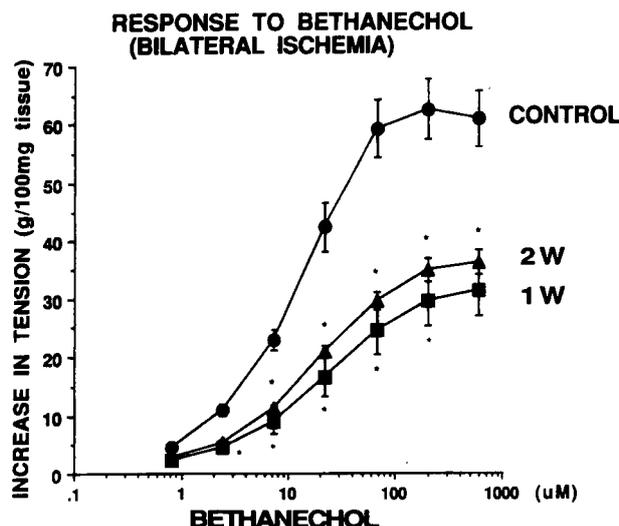


Fig. 5. Effect of bilateral ischemia on bladder responses to bethanechol. Each point is the mean \pm SEM of 6 duplicate observations. * Significant difference in difference from the control response, $p < 0.05$

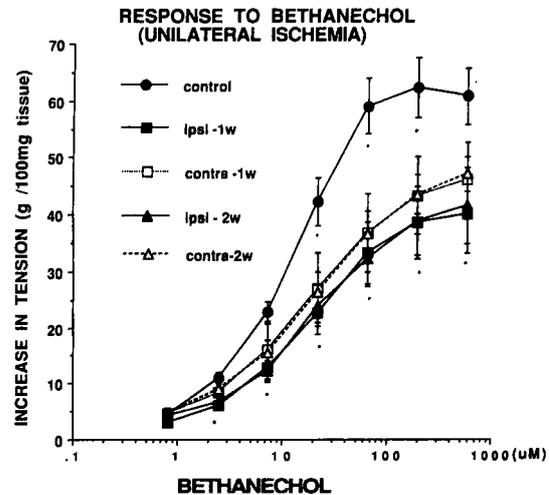


Fig. 6. Effect of unilateral ischemia on responses to bethanechol. Each point is the mean \pm SEM of 6 duplicate observations. * Significant difference from the control response, $p < 0.05$

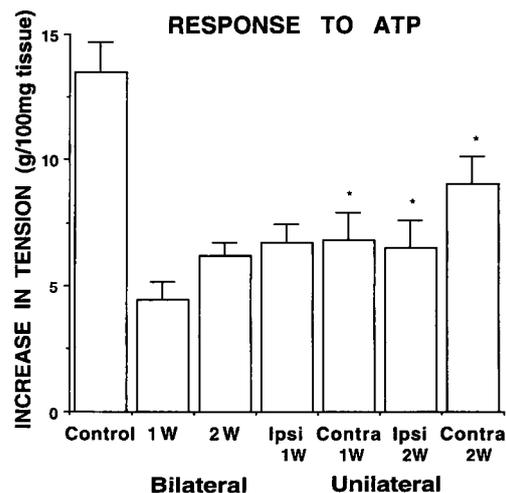


Fig. 7. The effect of bilateral and unilateral ischemia on the responses to ATP (2 mM). Each bar is the mean \pm SEM of 6 duplicate observations. * Significant difference from the control response, $p < 0.05$

lation (Fig. 3 and 4), bethanechol (Fig. 5 and 6), ATP (Fig. 7), and KCl (Fig. 8). There were no differences in contractile strength between muscle specimens harvested from the ipsilateral and contralateral sides of the unilateral ischemic bladders.

DISCUSSION

Circulation disturbances of the blood cause multiple organ failure of varying severity. Bladder ischemia may be caused by toxins, extensive electrocoagulation at TUR-P, pelvic thrombophlebitis, trauma, atherosclerosis, and unilateral ligation of the hypogastric artery at the time of renal transplantation^{2,3,5-9}

In the present study, bladder weight increased by bilateral and unilateral ligation of the internal iliac arteries for 1 week, which is consistent with the

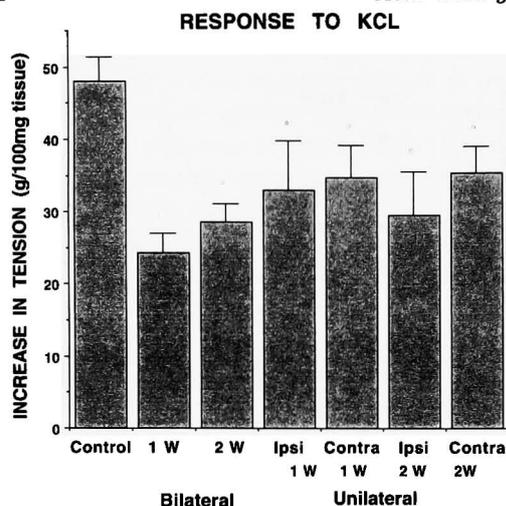


Fig. 8. Effect of bilateral and unilateral ischemia on the responses to KCl (124 mM). Each bar is the mean \pm SEM of 6 duplicate observations. * Significant difference from the control response, $p < 0.05$

findings in a rabbit model³⁾ We suggest that the significant increase in weight was due to the effects of inflammation and edematous change.

The increase in passive tension was significantly greater in the ischemic bladder. This observation is also consistent with previous findings in a rabbit model³⁾. The peak active tension occurred at a length of 16 mm in both the control and ischemic bladders. Passive tension at this length was calculated to be approximately 1.4 g, implying that putting 1 g of resting tension on the muscle specimens prior to any *in vitro* experiments is quite appropriate.

The contractile strength of bladders subjected to either unilateral or bilateral ischemia decreased in response to intramural nerve stimulation, direct receptor stimulation and depolarization of the smooth muscle. These findings suggest that impaired contractility induced by ischemia was caused by myogenic damage. Muscle specimens obtained from the left side of unilateral right-sided ischemic bladders showed the same impairment of contractility as specimens obtained from the right side. This finding suggests that some biological or metabolic alteration in the detrusor muscle, such as a decrease in cellular oxygen, together with edematous changes in the

intercellular space may have occurred on the contralateral side of the bladder. Lin et al. suggested that a decrease in the amount of glycogen in the ischemic detrusor was responsible for the impaired detrusor contractility in both the ischemic and contralateral sides of unilaterally ischemic rabbit bladders¹⁰⁾

In conclusion, bladder ischemia induced by ligation of the internal iliac artery impaired isolated detrusor contractility in response to intramural and pharmacological stimulation.

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(迅速掲載)

和文抄録

虚血（内腸骨動脈結紮）のラット膀胱機能におよぼす影響

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ラット膀胱機能に対する循環不全の影響を，内腸骨動脈の両側結紮および片側結紮の二つのモデルを作成して検討した．手術後1週目と2週目に膀胱を取り出して膀胱筋切片を作成して，電気刺激および各種薬剤に対する反応性をコントロールと比較した．両側結紮では電気刺激，Bethanechol，ATP，KClのすべてに対する収縮反応性は，術後1週目，2週目のいずれの膀胱とも有意に低下した．一方片側結紮では，虚血

側，反対側いずれの反応性もすべての刺激に対してコントロールに比較して有意に低下した．その程度は，両側結紮に比較して軽度であったが，虚血側と反対側で反応性に有意差を認めなかった．以上の結果より内腸骨動脈の両側又は片側結紮による虚血に伴いラット膀胱の収縮反応性は低下すると結論された．

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