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# Contractile properties of ureters from rats with infravesical urinary outlet obstruction

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**Abstract** Mechanical properties of ureters from rats with infravesical urinary outflow obstruction were studied in vitro. Urinary outflow obstruction was created by partial ligation of the urethra in female rats. After 10 days a marked hypertrophy of the urinary bladder and a dilatation of the ureters were observed. Proximal and distal segments of the ureters from these animals were isolated and mounted in a wire myograph for force registration. Comparisons were made with ureters from control rats. The ureters from the rats with urinary outflow obstruction exhibited a large increase in lumen diameter and an unchanged thickness of the muscle layer. These data suggest that the dilatation of the ureters is associated with growth of the smooth muscle in the wall. All ureter preparations were relaxed in normal physiological salt solution. When the extracellular $K^+$ concentration was increased to 20 mM the dilated ureters became spontaneously active. At $[K^+]$ in the range 20–40 mM in the presence of noradrenaline ($10^{-5}$ M) all ureters exhibited high-frequency spontaneous contractions. The dilated ureters had a lower frequency of spontaneous contractions and a higher force. The results show a pronounced remodelling of the ureter wall following infravesical outlet obstruction. The structural changes were associated with alterations in the contraction pattern of the preparations, most probably reflecting changes in the excitation-contraction coupling of the growing cells.

**Key words** Ureter · Urinary bladder · Smooth muscle · Hypertrophy · Rats

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## Introduction

The transport of urine from the kidney to the bladder in the ureter is, under normal conditions, achieved by peristalsis generated by its smooth muscle cells. Spontaneous contractions originating in the pelvi-ureteric junction or in the proximal part of the ureter produce the pressure wave transporting the urine bolus towards the bladder [14]. Ureteric obstruction can cause dilatation of the ureter. The properties of experimentally obstructed ureters have been studied in animal models [2]. Infravesical urinary outflow obstruction is a clinically important cause of ureteric dilatation, but animal models of this condition have, to our knowledge, not been investigated in much detail.

We have previously studied the properties of the urinary bladder in rats with experimentally induced infravesical urinary outlet obstruction. Following partial obstruction of the urethra the urinary bladder responds with a dilatation associated with growth of the smooth muscle cells in the bladder wall [13]. We have observed that this obstruction of the urinary flow is often associated with dilatation of the ureters. The aim of the present study was to characterize the mechanical properties of the dilated ureters in vitro. In order to obtain representative force recordings from the circularly arranged smooth muscle in the ureters of the rat we have applied to the ureter preparations a wire myograph technique originally introduced for the study of microvascular preparations [10].

## Methods

### Operative procedure and preparation

The experiments were approved by the local animal ethics committee. Urinary outflow obstruction was induced in female Sprague-Dawley rats, weighing about 220 g, by a partial obstruction of the urethra as described by Uvelius et al. [13]. The animals were anaesthetized with methohexital sodium (Brietal, Lilly) and via a lower abdominal incision a loose ligature was placed around the

proximal urethra. After 10 days the rats were killed by cervical fracture and the urinary bladders and the ureters were dissected. Segments of similar lengths (1–2 mm) were cut from the proximal (about 1–3 mm from the renal pelvis) and distal (about 1–2 mm from the urinary bladder) parts of the ureter.

### Mechanical experiments

The ureter preparations were threaded with thin stainless steel wires (diameter 40 μm) and mounted in a myograph similar to that described by Mulvany and Halpern [10] for use with microvessels. The isometric force was recorded with Grass FT03 transducers. The preparations were allowed to equilibrate for 30 min in $Ca^{2+}$-free normal Krebs (N-Krebs) solution (for composition, see below) and stretched to a length where passive tension was about 0.5 mN/mm, which in preliminary experiments seemed to be the optimal for active force.

On each preparation the contractile responses to $K^+$ and $K^+$ in combination with a supramaximal concentration of noradrenaline were recorded. The total KCl concentration was varied in the range 6 mM (present in N-Krebs) to 100 mM at constant $CaCl_2$ (2.5 mM). For each $K^+$ concentration investigated the following scheme was followed: (1) relaxation in $Ca^{2+}$-free N-Krebs for about 5 min; (2) incubation in a solution containing 2.5 mM $CaCl_2$ and the respective $K^+$ concentration for 5 min (the frequency and amplitude of the contractions being recorded during the last 2 min); (3) incubation for 5 min in the $K^+$ solution with $10^{-5}$ M noradrenaline added, contractile activity being recorded during the last 2 min. The protocol was thereafter restarted from step (1) above, for a new $K^+$ concentration. The length and diameter of each ureter segment were measured through a microscope with a calibrated eyepiece.

### Histology

At the end of the experiments the preparations were fixed in the apparatus by adding 2.5% glutaraldehyde to the N-Krebs solution. Sections were cut for light microscopy and stained with azur II methylene blue. The thickness of the smooth muscle layer in the ureteric wall was measured on micrographs of the sections.

### Solutions

The normal Krebs buffered solution (N-Krebs) contained (in mM): NaCl 122, KCl 6.0, $MgCl_2$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 15.5, $KH_2PO_4$ 1.2 and glucose 11.5. The solution was gassed with 96% $O_2$ and 4% $CO_2$ giving a pH of 7.4. All experiments were performed at 37°C. $Ca^{2+}$-free solution was made by omitting $CaCl_2$ when mixing N-Krebs. Different total $K^+$ concentrations were obtained by varying the concentration of KCl. Noradrenaline (Sigma) was added from a $10^{-3}$ M stock.

### Statistics

All values are given as the mean ± SEM. Statistical comparisons were made using Student's *t*-test for unpaired observations.

## Results

The 10-day period of partial urinary outflow obstruction resulted in a marked increase in the weight of the urinary bladder (from 68 ± 4 mg to 240 ± 40 mg, $n = 6$ for both). It could easily be observed, during dissection, that the ureters were distended in the rats with outflow obstruction.

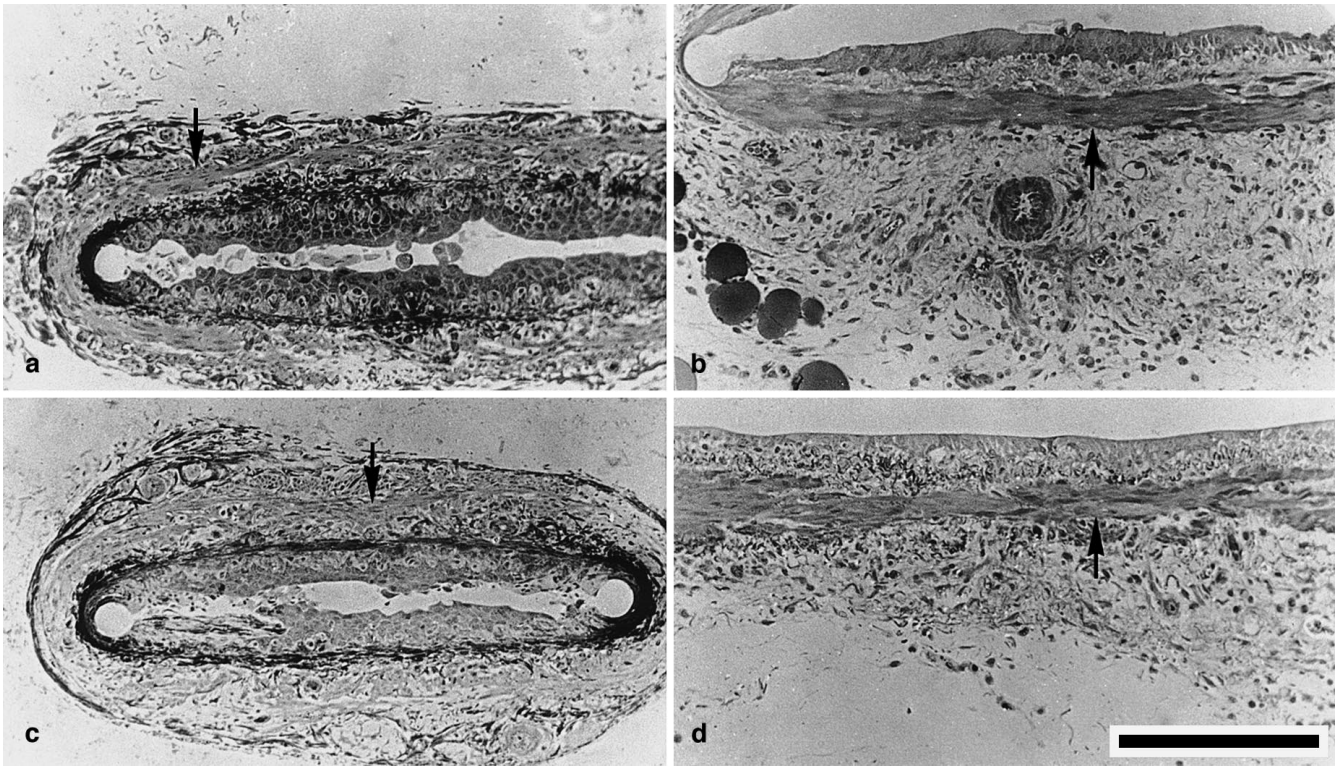


**Fig. 1** Light micrographs of proximal and distal ureter segments from a control rat (**a** proximal, **c** distal) and a rat with infravesical obstruction (**b** proximal, **d** distal). All pictures are at the same magnification. Note the marked increase in vessel circumference in the ureter from the rat with outlet obstruction. The smooth muscle layer in the wall of each preparation is indicated by an *arrow*. Scale bar represents 200 μm

Figure 1 shows light micrographs of the distal and proximal segments of ureters from a control rat and a rat with urinary outflow obstruction. Note the increased

**Table 1** Morphological data of ureters from control rats and rats with urinary outflow obstruction. For each preparation the circumference was measured during the isometric experiment using a microscope with an ocular scale. Muscle thickness in the wall was measured on the light micrographs. Muscle volume per segment length (i.e. cross-sectional area of the muscle layer perpendicular to the long axis of the vessel) was calculated as circumference multiplied by smooth muscle wall thickness. N-values are inserted in the table. Statistical comparisons were made, for corresponding segments, between ureters from controls and obstructed animals

| | Control | | Obstructed/dilated | |
|---|---|---|---|---|
| | Proximal | Distal | Proximal | Distal |
| Circumference (mm) | 2.0 ± 0.3 (5) | 1.9 ± 0.4 (5) | 10.0 ± 3.5 (5) $P < 0.05$ | 8.6 ± 2.2 (5) $P < 0.05$ |
| Muscle thickness (μm) | 58 ± 4 (7) | 70 ± 3 (6) | 51 ± 7 (5) NS | 55 ± 10 (5) NS |
| Muscle volume per segment length ($mm^2$) | 0.118 ± 0.017 (5) | 0.130 ± 0.041 (4) | 0.517 ± 0.229 (5) $P < 0.05$ | 0.414 ± 0.081 (5) $P < 0.05$ |

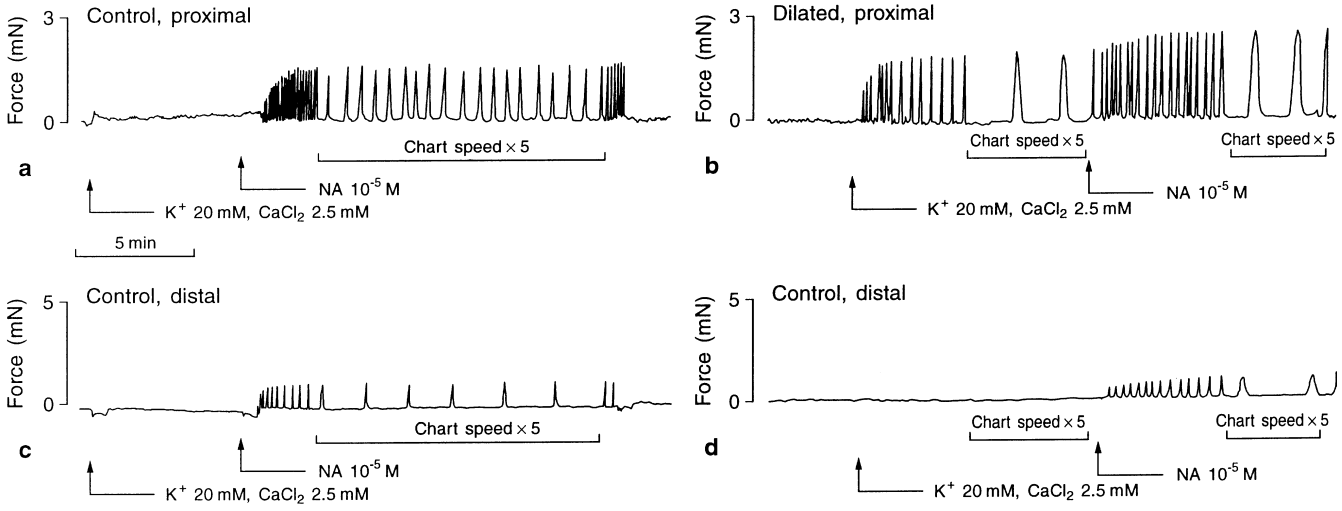


**Fig. 2** Original recording of force from proximal and distal ureter segments from a control rat (**a** proximal, **c** distal) and from proximal and distal segments from a dilated ureter from a rat with urethral obstruction (**b** proximal, **d** distal). When the muscles were transferred from the $Ca^{2+}$-free N-Krebs to a solution containing 2.5 mM $CaCl_2$ and 20 mM KCl the proximal segment from the dilated ureter developed spontaneous contractile activity. When $10^{-5}$ M noradrenaline was added all preparations became spontaneously active

circumference of the ureter from the rat with urinary outflow obstruction. In the wall, a circular layer of smooth muscle is observed between the basal membrane of the epithelium and the outer adventitia. The thickness of the muscle layer was not significantly altered in the dilated ureters.

The thickness of the muscle layer was measured on the light micrographs for each preparation and in Table 1 the morphological data are summarized. The ureters from the rats with urinary outflow obstruction had a similar smooth muscle wall thickness, a significantly larger circumference and a higher volume of smooth muscle per unit segment length (i.e. the cross-sectional area of muscle layer perpendicular to the long axis of the ureter, calculated as circumference multiplied by muscle thickness).

Original recordings of force from control and dilated ureters are shown in Fig. 2. All segments were relaxed in $Ca^{2+}$-free solution. At 20 mM $K^+$ with 2.5 mM $CaCl_2$ the proximal segment from the dilated ureter exhibited spontaneous phasic contractions (Fig. 2b). When noradrenaline was added all preparations became spontaneously active. The frequency of the contractions was higher in the proximal segments compared with the distal segments for both control and dilated ureters. The contraction frequencies of the distal and proximal segments in the dilated group were lower than those of the corresponding segments in the control ureter. The duration of the spontaneous contractions was slightly longer in the dilated ureters.

It can be seen in Fig. 2 that if rhythmic contractile activity was induced by $K^+$ and $K^+$ in combination with noradrenaline, it became stable within a few minutes. In the left-hand diagram of Fig. 3 the stable frequency of the spontaneous contractions is plotted against the $K^+$ concentration for the different ureter segments. The frequency of the spontaneous activity had an optimum around 20 mM $K^+$ for the proximal segments (circles). At 20 mM $K^+$ the proximal segments from the control and dilated ureters exhibited contractions with a mean frequency of about 5 $min^{-1}$. For the distal segments the optimal [$K^+$] appeared to be somewhat higher, at about 30 mM. As was noted in Fig. 2, not all preparations became spontaneously active at the optimal [$K^+$]. For the controls several preparations remained relaxed at 20 mM $K^+$ (cf. Fig. 2). For the proximal segments, four of eight controls and six of six dilated preparations were spontaneously active at

20 mM $K^+$. The corresponding numbers for the distal segments at 30 mM $K^+$ were: one of eight for the controls and two of six for the dilated preparations. At higher $K^+$ concentrations the preparations gave tonic responses or remained relaxed as described below.

The right-hand diagram of Fig. 3 shows the frequency of the spontaneous contractions in the presence of $10^{-5}$ M noradrenaline plotted against the $K^+$ concentration. All segments became spontaneously active when noradrenaline was added at $K^+$ concentrations in

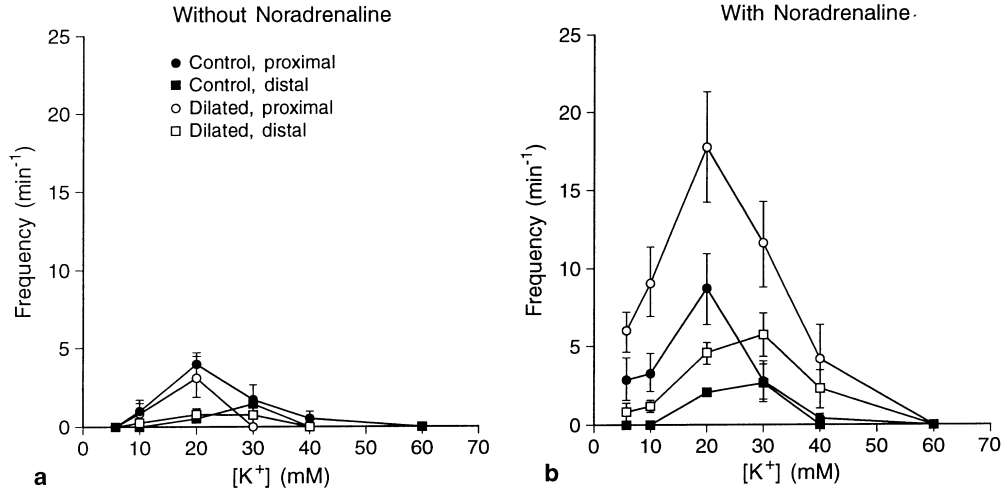


**Fig. 3** Frequency of the spontaneous phasic contractions at different $K^+$ concentrations in the absence of noradrenaline (**a**) and in the presence of $10^{-5}$ M noradrenaline (**b**). At intermediate $K^+$ concentrations in the presence of noradrenaline, where some preparations gave tonic responses, the data point shows the average of the frequency from the preparations exhibiting spontaneous activity ($n = 8$ and 6 for control and dilated ureters, respectively)

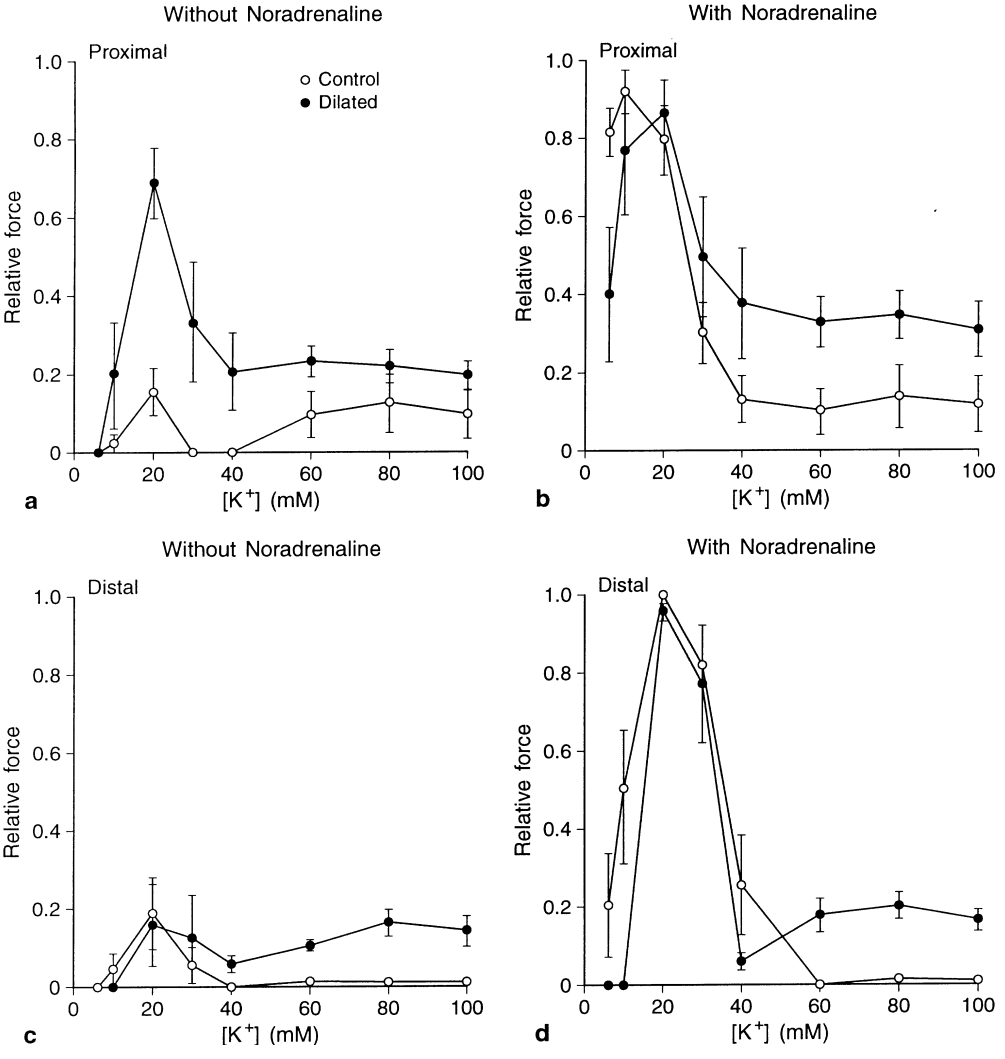


**Fig. 4a–d** Maximal active tension at different $K^+$ concentrations. The force values were recorded during the spontaneous contractile activity at lower $K^+$ concentrations and during the tonic contractions at higher concentrations. **a** Data from proximal segments; **c** data from distal segments obtained in the absence of noradrenaline. **b**, **d** Data with $10^{-5}$ M noradrenaline for proximal and distal segments, respectively. Force values for each preparation were normalized to the maximal active force obtained during stimulation with $K^+$ and noradrenaline. For control preparations tonic responses were found above 40 mM $K^+$. In the dilated group, both phasic and tonic contractions were found in proximal segments at 40 mM $K^+$ and at 30 and 40 mM $K^+$ in the presence of noradrenaline. In the dilated distal segments both types of contraction pattern were observed at 30 mM $K^+$ with or without noradrenaline. Above these concentrations all dilated preparations exhibited tonic contractions only ($n = 8$ and 6 for control and dilated ureters, respectively)

the range 20–30 mM. For both control and dilated ureters the frequency of the distal segments (squares) was lower than that of the proximal segments (circles) at each $K^+$ concentration. The maximal frequency was observed at a higher $K^+$ concentration in the distal segments. The maximal frequencies of contraction in the proximal and distal segments from dilated ureters were lower than those in the corresponding segments from control ureters.

In the left-hand panels of Fig. 4, the active force at different $K^+$ concentrations in the absence of noradrenaline is shown. The force values are normalized to the maximal active force recorded at optimal $K^+$ in the presence of noradrenaline for each preparation (see below). The $K^+$-induced force, relative to maximal, was lower in the distal segments (Fig. 4c). The right-hand panels (b, d) of Fig. 4 show the active force obtained at different $K^+$ concentrations in the presence of noradrenaline. For both control and dilated ureters the maximal force was obtained during the phasic contractions at $[K^+]$ in the range 20–30 mM. At higher $K^+$ (above 40 mM), tonic contractions were observed in the proximal segments of both types of preparation. These contractions had an initial force peak followed by a force decay to a stable plateau level. Force of the tonic contractions was measured at the plateau. In the control group, the proximal ureters gave tonic contractions with an amplitude of about 10% of maximal force at higher $K^+$ concentrations (above about 40 mM) whereas the distal ureters were relaxed. Addition of noradrenaline did not alter the force in these preparations at high $K^+$. In contrast, both the proximal and distal segments of the dilated ureters gave tonic contractions with an amplitude of about 15–20% at $[K^+]$ above 40 mM. The addition of noradrenaline resulted in an increase in tension.

Table 2 summarizes the active force output of the ureter preparations. The active wall tension and force per smooth muscle area were higher in the proximal segment of the dilated ureter. The average force value of the distal segment of the dilated ureter was also higher than in the corresponding control group, but the difference was not significant. Passive tension was $0.28 \pm 0.07$ and $0.78 \pm 0.034$ mN/mm ($n = 5$) for the proximal and distal segments of the control group respectively. The corresponding values for the dilated ureters were $0.41 \pm 0.13$ and $0.91 \pm 0.37$ mN/mm ($n = 5$). No significant difference was found.

## Discussion

The experimental infravesical urinary outlet obstruction in the rat was associated with a marked dilatation of the ureters showing that the distension present in the urinary bladder also extends to the ureters. Dilatation of ureters occurs in humans with different types of ureteric obstruction (cf. [15]). In previous work on experimental animals the ureteric dilatation has been created by ureteric obstruction [1, 2, 4, 5, 12]. Our experiments show that partial bladder outlet obstruction, which is associated with dilatation and growth of the bladder, is also associated with changes in the ureters. The structural changes with dilatation and hypertrophy of the bladder wall might contribute to increased ureteric pressure due to insufficient closure in the uretero-vesical junction. Also, the micturition pressure that the ureter might be exposed to in the obstructed bladder is increased [8].

Studies on rat ureter are sparse. This might reflect difficulties in performing mechanical experiments on vessels of this small size. With the use of the wire myograph technique we have obtained reliable force registrations from rat ureters. A detailed mechanical characterization of the rat ureters was, however, not possible since the muscles could not be activated to give reproducible tonic contractions. We could not, therefore, fully determine the shape of the length-tension relations in each preparation. The differences between the different groups were, however, found at all passive tensions applied. This suggests that they are not due to different degrees of stretch.

The force responses are dependent on the external $K^+$ concentration used to activate the vessel, and differences exist between different parts of the ureter. The ureters were normally relaxed in normal physiological solution but gave high-frequency phasic contractions when activated by $K^+$ and noradrenaline. Similar high-frequency spontaneous contractions induced by 40 mM $K^+$ have previously been reported for the guinea-pig ureter [7].

The thickness of the smooth muscle layer was unchanged in spite of the increased circumference at

**Table 2** Active wall tension and stress in ureters from control rats and rats with urinary outflow obstruction. The maximal active force was recorded for each preparation following activation with $K^+$ and noradrenaline ($10^{-5}$ M) as described in Results. Active wall tension was calculated from maximal active force and segment length. Active force per smooth muscle area (stress) was calculated from wall tension and the thickness of the smooth muscle layer. N-values are inserted in the table. Statistical comparisons were made between the dilated ureters and the corresponding segments in the control group

| | Control | | Obstructed/dilated | |
|---|---|---|---|---|
| | Proximal | Distal | Proximal | Distal |
| Active wall tension (mN/mm) | $0.54 \pm 0.09$ (6) | $0.64 \pm 0.22$ (6) | $0.91 \pm 0.12$ (5) $P < 0.05$ | $1.22 \pm 0.34$ (5) NS |
| Active stress per smooth muscle area ($mN/mm^2$) | $9.8 \pm 2.0$ (6) | $10.0 \pm 3.6$ (5) | $19.4 \pm 3.8$ (5) $P < 0.05$ | $21.8 \pm 5.7$ (5) NS |

similar passive force per segment length. This suggests that the smooth muscle layer had been extensively remodelled. Since the length of the ureter is constant, due to anatomical constraints, the increased smooth muscle volume per segment length of the dilated ureter suggests growth of the smooth muscle in the wall.

Previous work on obstructed ureters has shown that smooth muscle hypertrophy and/or hyperplasia occurs in the ureteric wall within days and can continue for several weeks after onset of obstruction [2, 4–6]. We have previously shown that growth of the urinary bladder in rats with infravesical urinary outflow obstruction is noticeable within less than a week [11, 13]. The present results show that the ureters have the capability for fast structural remodelling after onset of bladder outlet obstruction. We cannot, at present, determine whether the growth response involves hypertrophy, hyperplasia or a combination of these processes. In animals with longer periods of urinary outflow obstruction (6 weeks) we have observed that the ureters can be up to several millimetres in diameter (B. Uvelius et al., unpublished observations). The changes in ureteric structure observed after 10 days most probably reflect a relatively early change in the development of hydroureter and hydronephrosis.

The dilatation of the ureter was associated with changes in mechanical behaviour. The increased active wall tension might reflect an increased amount of smooth muscle in the wall. If the active force was normalized to the content of smooth muscle in the wall (active stress), higher values were found in the dilated group. This could reflect an alteration in the relative content of smooth muscle cells in the muscle layer, the orientation and mechanical coupling of the smooth muscle cells or the content of contractile components within the cells. It is also possible that the altered active force output reflects an alteration in the degree of activation of the preparations. The changed dependence on extracellular $K^+$ and the lower frequency of spontaneous contractions suggest that the excitability of the cells is altered in the dilated ureters. A decreased frequency of spontaneous contractile activity in normal physiological salt solution has also been described for growing vascular smooth muscle from the rat portal vein [9].

In conclusion, infravesical outlet obstruction in the rat was associated with an increased lumen diameter of the ureters. The volume of the smooth muscle layer in the ureteric wall increased with a corresponding increase in the active wall tension. Since wall stress (i.e. force per muscle area) also was increased, the remodelling of the ureteric wall might also involve a change in the relative content of smooth muscle in the muscle layer. As discussed above, the altered spontaneous frequency following activation, the altered duration of the spontaneous contractions and the altered responses to $K^+$ of the dilated ureters most probably reflect changes in the membrane properties of the growing smooth muscle cells. These changes appear to be associated with an increased excitability of the preparations and might be a factor contributing to the increased force-generating ability. These alterations in the smooth muscle physiology might be a consequence of the growth process itself, since changes in excitation-contraction coupling have been described for other types of growing smooth muscle cells [3, 9]. According to Laplace's law higher wall forces need to be generated to produce a pressure increase in a dilated vessel, and the increase in force-generating ability might be an important compensatory mechanism in rats with infravesical urinary obstruction. Further, an increased force-generating ability increases the contraction pressure when the ureter is closed, and thus increases the ability to maintain effective closure in the face of adverse pressure arising from downstream obstruction.

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