Urol Res (1998) 26:325–330

ORIGINAL PAPER

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Cystometrical evaluation of acute and chronic overdistension in the rat urinary bladder

Received: 19 February 1997 / Accepted: 18 September 1997

Abstract The urodynamic effects of an experimental, partial infravesical outlet obstruction in rats were studied and compared with the effects in sham-operated controls, and in animals that had undergone 24 h of total outlet obstruction. The animals were studied up to 42 days after surgery. Bladder weight increased with time in the partially obstructed group to reach a final value of 6 times that of the control. In water loading experiments micturition volume was unaffected by sham operation. In the partially obstructed bladders it decreased initially but normalized with time. In the group that had undergone 24 h of total obstruction micturition volume also decreased initially but then became significantly higher than in the controls. In cystometry experiments the partially obstructed bladders developed a considerable residual urine and increased threshold and micturition pressures. Detrusor instability was present already after 10 days. Also in the cystometry experiments the bladders that had been totally obstructed for 24 h had increased micturition volumes. Residual volume was only slightly affected by atropine in the control and partially obstructed bladders but increased 7-fold in rats in which the bladder had been totally obstructed for 24 h 42 days previously. We conclude that there is a close relationship between bladder weight, residual volume and micturition pressure in the partially obstructed bladder, and that 24 h of total obstruction results in disturbances of bladder function that might be related to denervation phenomena previously reported by others.

Key words Urinary bladder · Obstruction · Hypertrophy · Cystometry · Atropine · Rat

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Introduction

Experimental outflow obstruction in the rat has been shown to induce pathological changes in micturition pattern similar to those found in man [10]. After 6 weeks of partial obstruction a large bladder capacity, a high micturition pressure, a significant residual urine and a marked bladder instability develop. The increased pressure is due to the combination of an increased outlet resistance and the increased muscle mass in the bladder. The detrusor instability following outlet obstruction has been suggested to be due to a partial denervation of the bladder wall [3, 17]. Others argue, however, that the instability is of myogenic origin [5]. The original aim of the present study was to see how soon the observed changes in micturition pattern mentioned above develop, and how they change with time. Kitada et al. [6] have shown that 4–24 h of complete obstruction of the urethra resulted in a decrease in the contractile response to both muscarinic stimulation and direct muscle stimulation, as well as a decrease in muscarinic receptor density. Also, acute distension of the bladder wall has been shown to cause a transient nerve degeneration [7, 8]. What happens when the bladder is relieved of this obstruction in terms of micturition parameters? Some of our obstructed rats were, during postoperative care, found to be completely obstructed at 24 h after surgery. We decided to relieve them of the obstruction and follow them in the same way as was originally planned for the partially obstructed rats, to see how soon and to what extent bladder function is restored after 24 h of complete obstruction.

Materials and methods

A total of 79 Sprague-Dawley rats, initially weighing 225 g, were used in the experiments. The experiments were approved by the local Animal Ethics Committee.

Surgery

Partial bladder outlet obstruction was induced by exposing the bladder and proximal urethra through a midline incision and then tying a ligature of 4-0 silk around the proximal urethra and a rod of 0.9 mm diameter. The function of the rod was to obtain an equal degree of obstruction in all animals and it was removed after tying the ligature. For details on methodology see Mattiasson and Uvelius [13]. Methohexital sodium (Brietal, 50 mg/kg) intraperitoneally was used for anaesthesia. The animals were observed for 24 h after surgery. Most animals voided repeatedly during this period but some had no micturitions at all, indicating that they had developed complete urinary retention. In these cases the ligature was removed and the animal monitored in the same way as it would have been with a partial outlet obstruction. At each operation the abdominal wall was closed in two layers. Sham-operated animals, where the lower abdominal wall was incised and closed again, served as controls.

Water loading

Without anaesthesia a stainless tube was introduced through the mouth and into the stomach of the rat. Through this tube distilled water, amounting to 7% of the rat's body weight, was instilled. The animal was then placed in a metabolic cage. Micturition volumes and intervals were monitored for 120 min by means of a fluid collector connected to a Grass FTC03C force displacement tranducer and recorded by a Grass polygraph. When reading the graphs, no attention was paid to the first micturition, nor to the first micturition interval. Micturition volumes and intervals were measured, the maximal micturition volume established and the median micturition volume as well as the median micturition interval calculated. Normally rats have negligible diuresis during daytime, when the experiments were carried out. The water loading was performed on days 2 and 1 before surgery and days 1, 2, 3, 10 and 42 after surgery.

Cystometry

Through a midline incision of the abdominal wall a thin polythene catheter (Clay-Adams PE-50) with a cuff was inserted and sutured into the bladder and tunnelled subcutaneously to the back. Another catheter was introduced into one jugular vein to allow subsequent intravenous administration of atropine. The bladder catheter was implanted 2 days before cystometry to allow the bladder to accommodate, and the jugular vein catheter was inserted the day before cystometry. Methohexital sodium was again used for anaesthesia except in the group of animals that were totally obstructed and had cystometry 3 days after surgery, where ether was used. The animals were placed in metabolic cages and the bladder catheter was connected to a pressure transducer (situated at the level of the bladder) and an infusion pump (Microinject, Bioinvent). After the bladder was emptied via the catheter, a saline infusion at room temperature was instilled into the bladder at a rate of 10 ml/h. For details see Malmgren et al. [10]. Micturition volumes were monitored by the same procedure as in the water loading experiments. After 30 min, infusion was terminated immediately after a micturition and residual urine measured by lowering the tip of the bladder catheter below the level of the animal, allowing hydrostatic pressure to empty the bladder. One hour later, atropine at a dose of 1 mg/kg, demonstrated to be optimal for peripheral muscarinic blockade [11], was given intravenously and 10 min later the bladder infusion was started again and continued for 30 min. The cystometry experiments were performed in rats where partial obstruction or total obstruction for 24 h had been induced and in sham-operated animals 3, 10 or 42 days after surgery. After the experiment the bladders were dissected out and weighed.

The following urodynamic variables were measured and calculated:

Basal pressure: The lowest pressure during cystometry (in animals with zero residual volume this is the passive pressure in the empty bladder and in animals with residual volume it corresponds to the pressure at this volume)
Threshold pressure: Bladder pressure immediately before onset of micturition contraction
Micturition pressure: Maximum bladder pressure during micturition. As the rats do not seem to strain and have an abdominal pressure close to zero, micturition pressure is almost identical to the detrusor pressure [4]
Micturition volume
Residual volume
Residual volume after administration of intravenous atropine
Atropine resistance: Micturition pressure with atropine in relation to micturition pressure without atropine

Statistics

The results are given as mean value ± SE; the number of animals is also given. The rats with partial outlet obstruction and the rats with 24 h of total obstruction were compared with sham-operated controls at the same postoperative time using Student's *t*-test (two-tailed) for unpaired data. The Bonferroni method was used to compensate for the simultaneous comparison between more than two groups.

Results

Bladder weight increased more than 2-fold (Fig. 1) at day 10 and about 6-fold at day 42 in the partially obstructed rats, indicating a significant degree of obstruction. The weights of the bladders totally obstructed for 24 h increased slightly, but significantly, at days 3 and 10, but were similar to controls at day 42.

Water loading experiments

There was no significant difference in diuresis between the different groups during the 2-h water loading experiment. Median micturition interval was markedly shorter in both obstructed groups the first day after surgery. It then levelled off in the partially obstructed group to become approximately the same on days 10 and 42 as in the control group. In the group with 24 h of

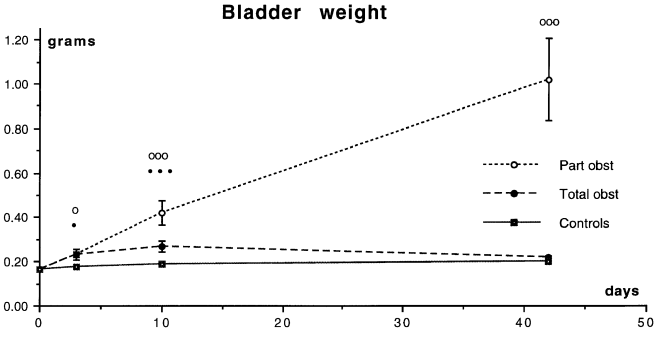

Fig. 1. Bladder weight versus time after surgery. The control (sham-operated) bladders (*filled squares*) have the same weight throughout the period. The bladders that were totally obstructed for 24 h (*filled circles*) had a transient weight increase, but returned then to control level. The partially obstructed bladders (*empty circles*) showed a steady weight gain throughout the period. The *symbols* above the curves show the degree of significant difference from the respective control group. Each group consists of 8–16 animals

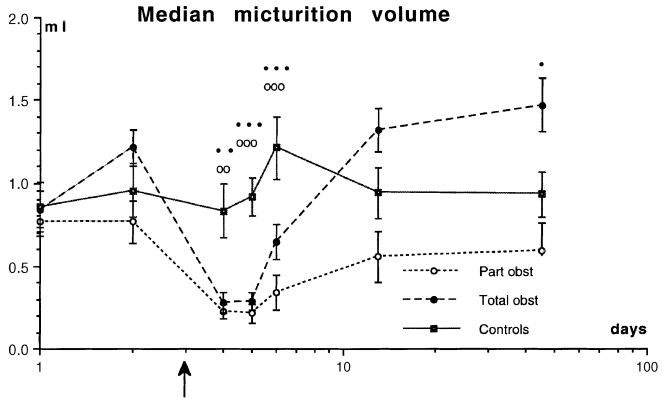

Fig. 2. Water loading experiments: median micturition volume versus time. The day of operation is indicated by the *arrow*. The control group (*filled squares*) was little affected by the sham operation, whereas both the group with partially obstructed bladders and the group with total obstruction for 24 h had a period with a significantly decreased micturition volume. Then this volume increased in the group with total obstruction, eventually reaching a value significantly higher than that of the control group. *Significance symbols* as in Fig. 1. Each group consists of 8–16 animals

total bladder obstruction, however, the intervals became increasingly longer and are highly significantly so at 10 and 42 days postoperatively. The mean micturition volume followed, as it should, the inverse pattern (Fig. 2), with a clearcut decrease in micturition volume at days 1, 2, and 3 after surgery for both obstructed groups. With time the micturition volume normalized for the partially obstructed group and increased to supranormal values for the totally obstructed group. The maximal micturition volumes followed the same pattern, though less pronounced.

Cystometry experiments

A typical cystometrogram of a control rat is shown in Fig. 3. Basal, threshold (Fig. 4A) and micturition (Fig. 4B) pressures rose approximately linearly with time in the partially obstructed group but remained low in the group with 24 h of total obstruction. Note that these pressures were measured after the first micturition and allow for residual urine to be present. As in the water loading experiments, micturition volumes were increased in the group with 24 h of total obstruction at days 10 and 42 (Fig. 4C). Residual volume (Fig. 4D) rose sharply and linearly with time in the partially obstructed group, was present in the group with 24 h of total obstruction at days 3 and 10, but was close to zero, as in the control group, at day 42. Detrusor instability was clearly seen in the 10 day and 42 day partially obstructed groups, but in no other groups.

Intravenous injection of atropine induced small but non-significant increases in residual urine in all groups at days 3 and 10. At day 42 the residual volume without atropine was 0.19 ± 0.07, 0.12 ± 0.04 and 7.50 ± 2.51 ml ($n = 9$ for all groups) for control, 24 h obstructed and partially obstructed bladders, respectively. The corresponding figures with atropine were 0.30 ± 0.09, 0.860 ± 0.093 and 8.12 ± 2.25 ml. The discrete increases for the control and partially obstructed groups were not significant. The 7-fold increase for the group with 24 h of total obstruction was, however, highly significant. Atropine resistance, expressed as micturition pressure with atropine divided by micturition pressure without atropine (Fig. 3), decreased with time of partial obstruction and at day 42 was significantly lower (40.0 ± 34.0%) than the corresponding figures for control bladders (67.0 ± 8.5%) and bladders with 24 h of total obstruction (75.4 ± 10.0%, $n = 9$ for all groups).

Discussion

The original aim of the present study was to evaluate how rapidly cystometrical changes develop when the bladder outlet is partially obstructed in the rat. Studies of this sort are, for obvious reasons, not possible in humans. Usually patients present with outlet obstruction of a considerable duration, and the urodynamic findings reflect a relatively late stage. As some of the animals developed a total obstruction, and therefore had the urethral obstruction removed after 24 h, the effects of acute bladder overdistension similar to that found in man was also studied.

We have previously found that experimental partial outlet obstruction in the rat bladder leads to a fast increase in bladder weight [20]. The final weight, which often exceeded the normal by a factor of 6–10, was

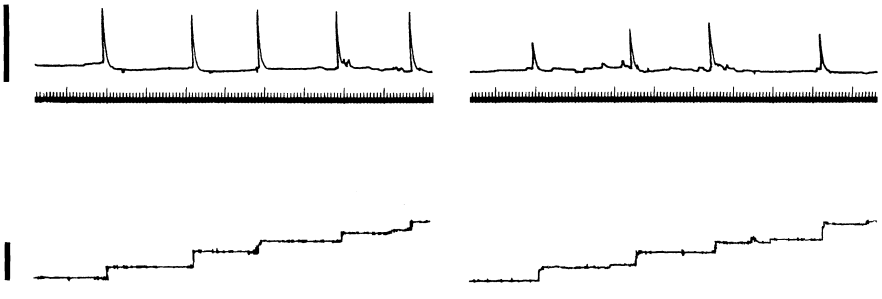

Fig. 3. Original cystometrical recording from a control rat 10 days after sham operation. *Upper tracing* shows bladder pressure (bar to the left, 100 cm H_2O), *lower tracing* micturition volume (bar to the left, 1 ml). Distance between tall bars on scale: 1 min. *Left panel* shows a recording before, and *right panel* a recording with atropine (see Materials and methods). There is a moderate decrease in micturition pressure following administration of atropine (see Results)

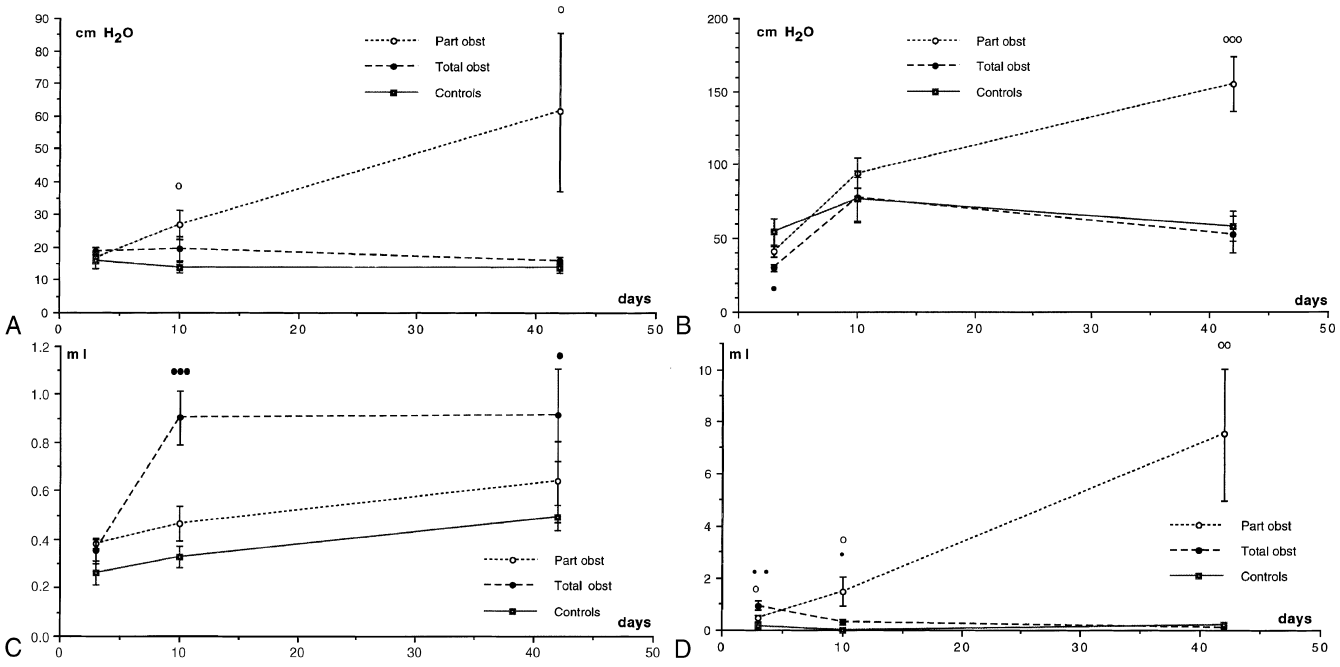

Fig. 4A–D. Cystometry experiments. **A** Threshold pressure. Note the steady increase for the partially obstructed group. **B** Micturition pressure. There is a transient decrease for the totally obstructed group, and a steady increase for the partially obstructed group. **C** Micturition volume. There was no difference between the control and partially obstructed groups, but a significant increase in the animals with total obstruction for 24 h. **D** Residual volume. The control bladders had almost no residual urine. There was a transient increase in the totally obstructed group, and a steady increase in the partially obstructed group. *Significance symbols* as in Fig. 1. Each group consists of 8–16 animals

reached after 6 weeks. The weight gain is to a considerable degree due to smooth muscle hypertrophy [2], although a net synthesis of collagen also occurs [20]. Cystometries performed at 6 weeks after onset of obstruction revealed a considerably increased micturition pressure, and the presence of detrusor instability [10].

In the present study we confirmed the excessive increase in weight of the partially obstructed bladder. Twenty-four hours of total obstruction induced a transient increase in weight, but we do not know whether this was due to an abortive growth of the bladder wall or, for example, edema secondary to the distension.

The mechanisms behind the detrusor instability induced by obstruction are not known with certainty. Steers and De Groat [18] suggested a neural plasticity in parasympathetic reflexes. Sibley [17] and Harrison et al. [3], on the other hand, suggested that the instability was related to a partial denervation of the bladder wall. There is also evidence for a myogenic origin; the instability was still present after administration of ganglionic blockers or tetrodotoxin [5]. The present study shows that the development of instability is rapid. It is found as early as 10 days after onset of obstruction when the bladder is still in a phase of rapid growth.

The water loading experiments showed that with time the bladders that had been totally obstructed for 24 h developed a micturition pattern with an increased interval between micturitions, and where each micturition had an increased volume. Acute distension induces a degeneration of both sensory and motor nerves in the rat bladder [7, 8, 16], but innervation density seems to have normalized after 3 weeks. The obstruction in the present study was, however, of a longer duration. Partial motor denervation would lead to a decreased micturition pressure [1]. The similar micturition pressure in control and totally obstructed bladders at days 10 and 42 in the present study is not supportive of a pronounced motor nerve denervation. The increased micturition interval could be explained by a lasting effect on sensory nerve function.

Control bladders had, in the cystometry experiments, a minimal volume of residual urine. Three days after surgery this volume had increased significantly in both obstructed groups. The increase was most pronounced for the group with 24 h of total obstruction. At 42 days the residual volume had normalized for this group. Contrary to this, the partially obstructed bladders developed with time an almost linear increase in residual urine. Thus there seems to be a direct relation between the development of residual urine and the increase in bladder weight.

The residual volume increased when atropine was given to the rats. The increase was low for the controls and the partially obstructed rats. For the latter group Malmgren et al. [11] found a more pronounced increase after atropine, but in their study residual volume without atropine was only about 50% of that reported here. We think that the bladders partially obstructed for 42 days in the present study are decompensated or are close to being so. It is possible that passive properties of the wall of the distended bladders put a mechanical constraint on the possible increase in residual volume

with atropine. We could confirm that atropine resistance of micturition pressure decreased [11] after partial obstruction.

There is an interesting difference between control bladders and those with total obstruction for 24 h at 42 days after surgery. Both groups had low residual volumes without atropine. With atropine, the residual volume of the controls was only slightly affected, but in the group with 24 h of total obstruction it increased 7-fold. Such an effect of atropine was found by Carpenter in rats after partial denervation of the bladder and the urethra. The increased effect of atropine might suggest a lasting disturbance in bladder and urethral innervation. In the bladder this is not apparent (see above) by light microscopy [7, 8].

The partially obstructed bladders were found to have slightly, though not significantly, lower micturition volumes than the controls. This is contrary to the increased mean micturition volumes found by Saito et al. [15]. The difference could be due to different experimental setups; they studied normal diuresis and we investigated the diuresis following a water load. Also, the obstruction was probably less severe in their study as the bladder weight increased only to about 200 mg.

Threshold pressure was the same in the control group and the group with 24 h of total obstruction. It increased, however, with time in the partially obstructed bladders and thus seems to be directly related to the increases in residual volume and bladder weight. Micturition pressure depends on outlet resistance, force produced by the detrusor muscle cells and, according to Laplace's law, the degree of distension. It is interesting to note that although the partial obstruction would be expected to increase outlet resistance immediately, there is no immediate increase in micturition pressure. Instead micturition pressure increased with time during the observed period (3–42 days after surgery). One plausible explanation is that outlet obstruction slowly increases with time due to, for example, fibrosis around the ligature. Another explanation is that the contractile strength of the detrusor muscle cells increases only slowly with time, for example as a consequence of synthesis of contractile proteins [12].

The time-dependent increase in basal pressure in the partially obstructed group is probably due to the increased residual urine, as in these animals basal pressure is the pressure at this volume (see definitions of measured variables in Materials and methods).

The totally obstructed bladder had a decreased micturition pressure 3 days after surgery. This could be a consequence of nerve degeneration in the bladder [7, 8, 16] and disruption of junctions between the smooth muscle cells [9]. Injury to the nerves innervating the urethra with a resulting subnormal outlet resistance is also possible, but seems less likely. In man, acute distension has been reported [14] to have a pronounced and lasting effect on the detrusor muscle cell structure, with a limited functional recovery.

We did not find any evidence for lasting dysfunction of the detrusor muscle cells in the totally obstructed bladders; after 42 days micturition pressure was similar to that in the controls (note, however, the increased response to atropine described above). Also, Sehn [16] could not detect any lasting ultrastructural effect on the smooth muscle cells after 6 h of severe overdistension. Overdistension causes a proliferative reaction involving urothelium, smooth muscle and connective tissue [19]. Our results suggest that the detrusor muscle has a considerable capacity to recover functionally after overdistension.

Acknowledgements The study was supported by grant no. 14x-9479 from the Swedish Medical Research Council, and the Medical Faculty, Lund University. We are grateful for the technical assistance of Ms. Magdalena Dawisciba.

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