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Effect of aging on bladder function and the response to outlet obstruction in female rats

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Abstract Bladder dysfunction in the aging population is a significant problem. However the concomitant presence of other diseases in many patients can make it difficult to distinguish between changes in bladder function and other influences. The present study was designed to study, in aging rats, bladder function and the effect of partial bladder outlet obstruction (BOO) on bladder function. Cystometrics were performed in awake, female Fischer 344 rats of four age groups (6, 12, 18 and 24 months) following subcutaneous implantation of a mediport catheter. Cystometric evaluations were carried out in control rats or those subject to three weeks of BOO. Bladder compliance significantly decreased with aging, which reflected an increase in threshold pressure without changes in bladder capacity. Partial BOO caused development of severe bladder instability. Following BOO, bladder capacity and compliance were significantly increased in all age groups. Threshold pressure was lower in obstructed animals, except for 6-month rats. Younger animals were able to generate a higher contraction pressure to compensate for the BOO, whereas older animals did not. Using an awake model of cystometric measurement, we have demonstrated that aging, by itself can affect bladder function. Furthermore, aged animals respond differently to BOO than younger animals. These results demonstrate that both aging and disease can contribute to bladder dysfunction, and suggest that treatment of bladder dysfunction may require a combination of therapies targeted to multiple etiologies.

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Introduction

The urinary bladder serves to collect and store urine at low pressure and to periodically expel its contents via a sustained synchronous contraction of the detrusor muscle [4, 20, 25]. As people age, bladder dysfunction is a common complaint. Benign prostatic hypertrophy (BPH) affects up to 70% of the elderly male population in the United States [8]. Approximately 30% of the elderly living in the community and 40% of institutionalized elderly are chronically incontinent [17]. Elbadawi et al. [5] reported the findings of their urodynamic examination of a group of elderly patients (a) detrusor overactivity, 43% (b) outlet obstruction, 11% (c) a combination of both overactivity and obstruction, 9%, and the remainder showed neither obstruction nor overactivity.

Chun et al. [1] noted that older male rats (22–24 months of age) had increased water intake and increased urine output as compared to younger rats (5–7 months of age). The pressure at micturition was greater in older rats, and there was no age-related difference in the bladder volume at micturition. Italiano et al. [9] noted that threshold volume and threshold pressure were increased in older males compared to younger rats. There have been no cystometric studies of aging female rats. Bladder outlet obstruction (BOO) in humans may result from BPH, urethral stricture disease, or congenital anomaly [25]. The functional changes that develop in response to obstruction include detrusor instability, elevated contraction pressures, and the presence of residual urine [24]. Such findings have been documented in several species using different methods to evaluate bladder function [10, 11, 15].

In the present investigation, we were interested in studying bladder function in female rats of four different ages, along with studying how the aging bladder

responds to BOO. These studies have not been carried out before, in either male or female rats. Using the method of awake cystometrics, we have been able to obtain information on both the filling and voiding cycles in both obstructed and unobstructed rats. Since BOO is such a prominent finding in older patients, such studies should provide insight into the interaction of aging and obstruction.

Materials and methods

Animal model

Female Fischer 344 rats (270–350 g) of four age groups were used. Both control rats and those subjected to 3 weeks of partial outlet obstruction were studied. Female rats were used, since it has been reported that outlet obstruction of male rats causes reflux of urine into the seminal vesicles and coagulating glands, effectively decompressing the bladder [21].

Cystometric evaluation

Cystometrics were performed in awake animals using a technique previously described [16]. Briefly, a subcutaneous pocket was created in the posterior cervical area and a mediport catheter (Norfolk Medical, Skokie, Ill.) was placed within the pocket and its catheter end subcutaneously tunneled and introduced into the bladder via the dome. The following day, cystometric studies were performed by infusing saline into the bladder via the mediport catheter, which was connected to both an infusion pump and a pressure transducer (Statham P23 XL, Gould-Statham, Oxnard, Calif.). The infusion rate was 0.052 ml/min and 0.1 ml/min for the unobstructed rats and obstructed rats, respectively. Figure 1 shows a typical tracing of a cystometric evaluation. The following parameters were calculated (a) bladder capacity (voided volume + residual volume), (b) threshold pressure (premicturition pressure – pressure at start of infusion), (c) compliance (bladder capacity/threshold pressure), (d) percent void (voided volume/ bladder capacity (*100), (e) contraction pressure (maximum pressure – premicturition pressure), spontaneous activity (frequency of pressure fluctuations in a 2-min interval prior to micturition). For each animal, three to five repeat cystometric evaluations were obtained, and there were three to four rats per group for each of the eight groups of rats.

Surgical creation of partial outflow obstruction

Animals were anesthetized with sodium pentobarbital (20–30 mg/kg) intraperitoneally. Via a low midline incision, the bladder and proximal urethra were exposed. A 4–0 silk ligature was placed around the urethra and tied loosely in place in the presence of a plastic rod (Clay-Adams PE-50, VWR Scientific, West Chestes, Pa.) with an external diameter of 0.96 mm. The plastic rod was then removed and the abdomen closed.

Statistics and calculations

Evaluation of the statistical significance was performed by means of analysis of variance (ANOVA), with $P < 0.05$ being considered significant. Data are presented as means ± SEM.

Results

Bladder function in aging rats

Bladder function was studied in four age groups categorized as (a) young – 6 months, (b) young adult – 12 months old, (c) mature adult – 18 months old, (d) old or senescent – 22–24 months old [14]. The present study utilized awake cystometry to monitor both the filling

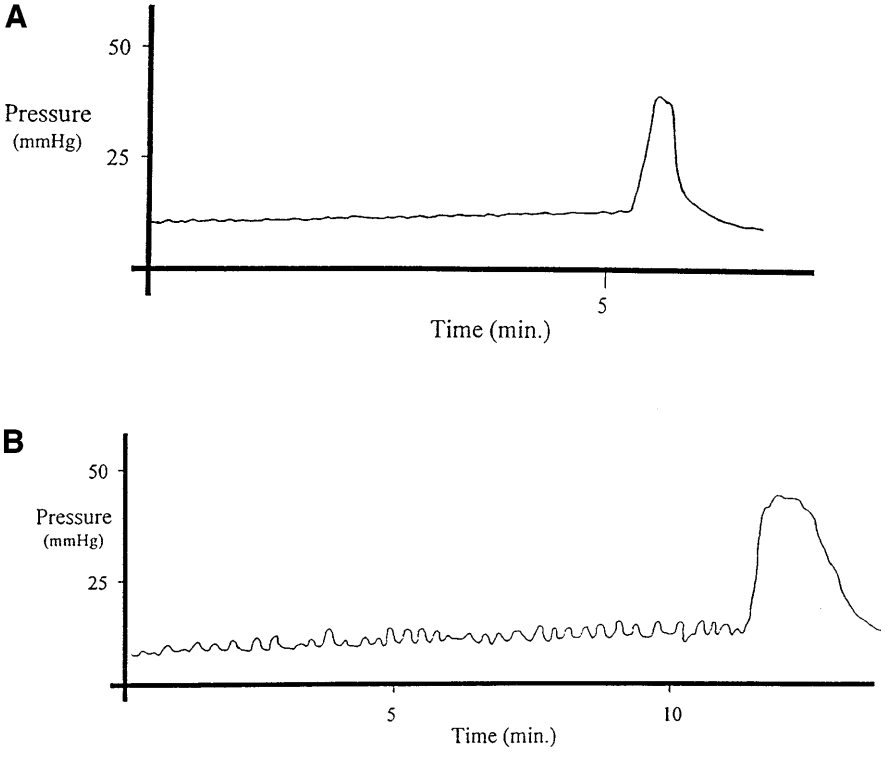

Fig 1a, b Cystometric tracing from a representative 12-month rat. **a** Sham-operated control. **b** Twenty-one days post bladder outlet obstruction (BOO). Cystometrics were carried out in awake animals as described in "Methods." Note the spontaneous contractions in the obstructed animal, which are not present in the control

and voiding cycles of micturition. A representative cystometrogram from a control 12-month rat is shown in Fig 1a. It can be seen that the bladder fills under low pressure and that the animal generates a sharp detrusor contraction. Spontaneous bladder contractions are not seen. There were no significant differences in bladder capacity, voided volume or residual volume among any of the ages examined (Table 1). Animals voided nearly to completion with the percent void greater than 80.0%. Compliance was significantly decreased in animals as they aged, with bladders of 6-month animals exhibiting a compliance of 0.22 ± 0.01 ml/cm H_2O compared to 24-month animals with a compliance of 0.10 ± 0.03 ml/cm H_2O. This was largely reflective of the increase in threshold pressure that was increased in all three of the older age groups.

Bladder function in aging obstructed animals

Animals of all four age groups were subject to 3 weeks of BOO described in "Methods." A typical cystometrogram of a 12-month rat obstructed for 3 weeks is shown in Fig 1b. As compared to the control 12-month rat, it can be seen that outlet obstruction is associated with the development of bladder instability, reflected in the development of spontaneous contractions in all obstructed animals studied. It is also noted that the detrusor contraction is broader than in the unobstructed animals. In obstructed animals, the number of spontaneous contractions recorded in the 2 min prior to micturition ranged from 16.1 ± 4.8 in 24-month animals to 37.0 ± 9.2 in 18-month animals.

An increase in bladder capacity was found after BOO in rats of all ages (Table 2) . Micturition volume was significantly increased only in 12-month animals. Conversely, there was a significant increase in residual volume in all age groups, except the 12-month animals. This was reflected in the percent void, which in control rats exceeded 80% for all age groups, but in obstructed rats was less than 50% in all age groups.

The threshold pressure was significantly lower in obstructed animals as compared to controls for all age groups, except for 6-month rats (Table 2). The compliance of the obstructed bladders was higher in all groups than in the control rats; compliance in the 24-month BOO rats was significantly lower than in all other groups. Contraction pressure was significantly higher in the 6-month group only (49.7 ± 19.9 cm H_2O compared to 6.3 ± 1.1 cm H_2O).

Discussion

Bladder dysfunction in the aging population is a significant problem. The concomitant presence of other diseases, however, in many patients [17] can make it difficult to assess changes in bladder function separately from other influences. In the present study we used a model of awake cystometrics to study bladder function in the Fischer-344 rat. These rats have been used extensively in many studies on aging. We found that bladder compliance decreases with aging. When animals of different ages were subject to 3 weeks of partial BOO, both old and young animals showed an increased bladder capacity and compliance and the development of spontaneous bladder contractions (bladder instability). The percent void decreased from an average of 82% in the control animals to 41% in obstructed animals. Contraction pressure in response to BOO was increased only in the 6-month animals. These results suggest that aging, by itself, can influence bladder function. Furthermore, the response to BOO in animals of different ages is not uniform and the aging process may contribute to bladder dysfunction in the elderly. Such effects may be more pronounced if other disease states are also present.

Table 1 Effect of age on bladder function in rats (*$P < 0.05$ compared to 6-month group)

Age	Bladder capacity (ml)	Residual volume (ml)	Percent void	Threshold pressure (cm H_2O)	Voiding pressure (cm H_2O)	Compliance (ml/cm H_2O)
6 Months	0.39 ± 0.03	0.06 ± 0.00	83.9 ± 0.8	1.93 ± 0.16	6.3 ± 1.1	0.22 ± 0.01
12 Months	0.66 ± 0.11	0.11 ± 0.00	81.1 ± 2.64	4.44 ± 0.52*	6.53 ± 2.9	0.15 ± 0.01*
18 Months	0.59 ± 0.20	0.11 ± 0.04	82.5 ± 1.9	4.64 ± 1.06*	10.5 ± 6.3	0.12 ± 0.01*
24 Months	0.36 ± 0.02	0.06 ± 0.00	82.3 ± 0.91	4.85 ± 1.02*	10.1 ± 3.6	0.10 ± 0.03*

Table 2 Effect of bladder obstruction on bladder function in rats of different ages (*$P < 0.05$ vs. same age animal in control group $^{\#}P < 0.05$ vs. 24-month obstructed rat)

Age	Bladder capacity (ml)	Residual volume (ml)	Percent void	Threshold pressure (cm H_2O)	Voiding pressure (cm H_2O)	Compliance (ml/cm H_2O)
6 Months	1.98 ± 0.57*	1.32 ± 0.74*	46.7 ± 17.2*	1.8 ± 0.62	49.7 ± 19.9*	1.13 ± 0.07*$^{\#}$
12 Months	2.46 ± 0.29*	1.28 ± 0.25	49.5 ± 4.5	2.1 ± 0.2*	22.3 ± 2.1	1.14 ± 0.03*$^{\#}$
18 Months	2.36 ± 018*	1.51 ± 0.19*	36.4 ± 5.2*	2.1 ± 0.18*	22.2 ± 11.9	1.18 ± 0.01*$^{\#}$
24 Months	2.27 ± 0.15*	1.56 ± 0.09*	30.1 ± 7.7*	2.9 ± 0.78*	22.3 ± 12.2	0.92 ± 0.12*

We recently reported on our use of an in vivo cystometric system to monitor bladder function in awake, neurologically intact animals; this system eliminates the use of anesthesia, which depresses spontaneous contractile activity and the micturition reflex [13, 16]. Using this technique in the present study, we noted no significant differences in either the bladder capacity, residual volume or contraction pressure in rats of the four different age groups. Chun et al. also found that there were no age-related differences in the bladder volume at micturition [1]. We did find a decrease in bladder compliance with aging, resulting from the significant increase in threshold pressure associated with aging. These results confirm the results of both Italiano et al. [9] and Chun et al. [1] who measured the threshold pressure in older and younger male rats. Since the present study used female rats, this suggests that similar changes take place irrespective of gender in the functional response of the bladder to aging.

The fact that a higher threshold pressure in required in the presence of the same bladder capacity in older rats as compared to young ones suggests that there may be a hyposensitivity to intravesical volume changes in the aging rat bladder. These findings are similar to those of Santicioli et al. [19] in their studies on capsaicin and bladder function. Capsaicin, a pungent component of Hungarian red peppers, is a neurotoxin specific to C Type primary afferent fibers [23]. In their studies, Santicioli et al. [19] determined that pretreatment of rats with capsaicin significantly increased the micturition threshold without altering the amplitude of the micturition contractions. Thus, alterations in the afferent pathways, similar to those produced by the pharmacologic effects of a neurotoxin, may be associated with aging.

Several investigators have studied the in vitro response of detrusor muscle strips from 6- and 24-month animals. Saito et al. [18] found that the contractile response to agents including acetylcholine, prostaglandin $F_{2\alpha}$, angiotensin II and vasoactive intestinal polypeptide was unchanged; the response to norepinephrine, ATP and serotonin was increased, and the response to Ca^{++} was significantly decreased. Munro and Wendt [15] demonstrated that at the same dose of carbachol, there was less force generated in detrusor muscle strips of aged rats than in younger ones; however, responses to either electrical stimulation or calcium were equivalent in older and younger rats. Using isolated whole bladder, Chun et al. [2] found no change in response with aging to several agents including bethanecol, phenylephrine, serotonin and histamine. Conflicting data may be the result of the types of preparation used or may reflect species differences, since three different strains were used by the three groups. Nevertheless, the above results, all obtained using in vitro preparations, demonstrate that the responsiveness of bladder tissue appears to be changed in aging, but such changes may not be consistent for all putative transmitters. Since bladders of older patients may exhibit both hyperactivity and impaired contractility [5], it may not be surprising that the response to various contractile agents is different.

Despite previous studies on aging rats, there have been no other reports on the effects of aging on the response to obstruction. We examined both volume and pressure parameters in obstruction in this study. In response to obstruction, there was a significant increase in bladder capacity in all age groups. Residual volume was increased and percent void was decreased in the 6-, 18- and 24-month rats. The pressure parameters appeared to be more consistently related to changes in age. Compliance was increased significantly in all age groups following obstruction. Despite the increase in compliance, the bladders of older animals exhibited less compliance than that of the other age groups. This is consistent with the findings in nonobstructed rats where the oldest animals had the lowest compliance. Furthermore, this finding is supported by studies on the aging human bladder which show trabeculation of the bladder and increased collagen, which could lead to decreased compliance [6, 12, 22]. Interestingly, a study by Gosling [7] examining bladder structural changes found that aging alone was associated with an increase in the collagen surrounding smooth muscle bundles in older patients without evidence of obstruction; however, such changes were not as marked as those seen in obstructed bladders.

Obstruction is more prevalent clinically in male patients; however, the present studies were carried out with female rats, since obstruction of male rats causes reflux of urine into the seminal vesicles and coagulating glands, which effectively decompresses the bladder. We did not examine the hormonal status of the female rats, nor did we carry out hormone ablation and replacement studies, as of yet. It is possible that hormones could have an effect on the bladder, both in aging and its response to obstruction. There have been few studies on the influence of gender on bladder function. Eika and colleagues [3] studied micturition and bladder strip contraction in age matched (although not specified) male and female rats. There was very little difference in control females compared to control males, in either micturition characteristics or in the response of bladder strips to either field stimulation or contractile agents. Neither ovariectomy nor castration had any effect on micturition characteristics, and castration had no effect on the response of bladder strips. In female rats, ovariectomy was associated with decreases in the response of bladder strips to field simulation, but not to carbachol, ATP or KCl. In vivo cystometrics were not investigated in their study. The preceding suggests that the hormonal milieu may not be critical for bladder function and that our results may be applicable to male bladders as well.

Threshold pressure in response to obstruction was decreased in the 12-, 18- and 24-month rats, whereas in 6-month rats there was no change compared to unobstructed controls; conversely, contraction pressure was significantly increased in the 6-month rats compared to controls, whereas there was no significant difference in the older animals. This suggests that younger animals

can adapt to obstruction better than older animals. They can maintain the threshold pressure found prior to obstruction and can generate a higher contraction pressure compared to their unobstructed controls, or older rats. In the short-term obstruction experiments carried out herein, this does not lead to a more efficient voiding, since the residual volumes were not significantly lower in younger animals. However, it remains to be seen if younger animals which can generate a higher contraction pressure would adapt better to a more chronic obstruction.

In summary, implantation of a subcutaneous mediport allows accurate cystometric evaluation to be performed in an awake animal without the depressive effects of anesthesia. Using this technique, we demonstrated decreased bladder compliance and increased threshold pressure in aging female rats. Partial BOO of 3 weeks' duration results in increased bladder capacity and compliance, and the development of bladder instability as seen by spontaneous contractions on cystometrics. Aged animals were not able to fully compensate for the BOO by increasing their contraction pressure. These results demonstrate that both aging and disease can contribute to bladder dysfunction, and suggest that treatment of bladder dysfunction may require a combination of therapies targeted to multiple etiologies.

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