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Functional and morphological modifications of the urinary bladder in aging female rats

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Functional and morphological modifications of the urinary bladder in aging female rats. *Am J Physiol Regulatory Integrative Comp Physiol* 278: R964–R972, 2000.—In female Wistar/Rij rats, 10 and 30 mo old, the micturition profiles in conscious animals, the contractile responses of the isolated urinary bladder, and the histology of the vesical tissue have been investigated. During cystomanometry, 60% of conscious senescent rats, but only 25% of young adult rats, showed spontaneous contractions during the bladder-filling phase. In aging rats, micturition pressure and duration of micturition were significantly higher by ~40–50%. In contrast, bladder capacity, bladder compliance, micturition volume, and residual volume were not modified with age. In vitro, the contractile responses of the bladder body to KCl, carbachol, arecoline, and α, β -MeATP were similar in tissues from young adult and senescent rats. In contrast, maximum responses to noradrenaline, but not phenylephrine, were two times greater in the older rats. Isoprenaline exhibited the same potency in relaxing KCl-precontracted bladder body of 10- and 30-mo-old animals. Morphometric analysis showed a significant increase in the mean thickness of the muscularis layer with age, whereas the collagen density significantly decreased in the muscularis and in the lamina propria layers. The fact that the majority of senescent rats displayed bladder instability and increased response to α -adrenergic agonists suggests that this strain of rat seems a good model for the aged human. However, other characteristics of the aging human urinary tract (urinary frequency, decreased cystometric capacity, and decreased detrusor contractility associated with fibrosis) are not present.

aging; bladder base; cystometry; urodynamics; Wistar/Rij rats

IT IS WELL KNOWN THAT DISTURBANCES of the urinary bladder function are common in elderly people who often complain of urgency, frequency, nocturia, and incontinence (30, 33). Age-related changes in the lower urinary tract functions include decreased bladder capacity, increased detrusor instability, and, particularly in women, decreased urethral resistance (4, 9, 33, 36).

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To date, the effect of age-related changes in urinary bladder function in humans is not well characterized because it involves invasive procedures, such as catheterization for urodynamic studies and cystoscopy. Moreover, in practice, it is difficult to precisely discriminate between dysfunctions belonging to pathology from those pertaining to aging. The complexity of studies in human aging is complicated by associated diseases and drugs that may affect the lower urinary tract.

The normal functions of the urinary bladder are storage and expulsion of urine (micturition). It is thought that the sympathetic nervous system exerts a tonic influence on the bladder and urethra that facilitates the storage by predominantly stimulating α -adrenergic receptors in the bladder base and urethra. At the same time, sympathetic stimulation of predominantly β -adrenergic receptors in the bladder body causes detrusor muscle relaxation. Micturition reflex manifests principally through activation of a cholinergic pathway on the bladder, but a purinergic system has also been described (3).

Several factors have been put forward to explain the voiding dysfunctions observed in the elderly, including detrusor fibrosis and consequent impairment of contractility (21), deposition and cross-linking of collagen and elastin (12), and loss of acetylcholinesterase-positive nerves (10). Despite the extensive use of the rat in aging research, the effect of senescence on rat urinary bladder function is not well characterized. Studies on the rat isolated detrusor muscle are numerous (18, 20, 22, 24, 25, 27, 28, 34, 41), but in vivo studies are scarce (5, 6, 16), and to our knowledge, there is no report on the effects of aging on bladder function in conscious animals.

We have therefore studied changes in micturition profiles in conscious young adult and senescent animals and the pharmacological responses to various agonists both in vitro and in vivo. An additive histological and morphometrical study of the bladder, using the same strain of rats, has been included.

MATERIAL AND METHODS

Animals

Young adult (10 mo old) and senescent (30 mo old) virgin female Wistar WAG/Rij rats were obtained from the specific pathogen-free husbandry of the Centre d'Etudes de Saclay (Gif sur Yvette, France). The mean survival time of individu-

als in this colony was ~30 mo. They were fed ad libitum and had free access to water. All experiments were performed in accordance with the guidelines for animal experiments and principles for the care and use of laboratory animals established by Synthelabo Research Ethical Committee following the national and international directives.

Cystomanometry in Conscious Animals

Surgical procedure. Rats were anesthetized with ketamine (Imalgene, Rhône Mérieux, France), 100 mg/kg body wt ip. The abdomen was opened through a midline incision, and the bladder was exposed. A polyethylene catheter (Merk Biotrol, E03403) was implanted in the bladder through the dome and exteriorized at the scapular level. Each rat was housed individually after surgery, and food and water were given ad libitum. Animals were allowed to recover for at least 48 h.

Cystomanometric recordings. Cystomanometric investigations were performed in conscious animals 2 days after the bladder catheter implantation in young adult and senescent rats, as previously described (23). The bladder catheter was connected via a T tube to a strain gauge and an injection pump (Harvard apparatus) (22). The rats were held under partial restraint, and a vial was placed under the animals to measure volume of urine expelled. Warmed saline (37°C) was infused into the bladder at a rate of 6 ml/h.

Intravesical pressure was continuously recorded using a Maclab/8e interface (AD Instruments) and Chart software (version 3.4.2). Data were analyzed with Microsoft Excel software on Power Macintosh. The following cystomanometric parameters were measured or calculated (Fig. 1): basal pressure (BaP, cmH₂O), peak micturition pressure (MP, cmH₂O), threshold pressure (ThP, pressure at which micturition occurs, cmH₂O), micturition duration (D, s), interval (time between 2 subsequent micturitions, min), micturition volume (MV, ml), residual volume (volume infused minus MV, ml). Five reproducible micturition cycles were analyzed, and means of the different cystometric parameters were calculated.

Bladder Contractility in Anesthetized Animals

Experiments were performed in young adult and senescent female Wistar/Rij rats. Under anesthesia (pentobarbital 30 mg/kg ip and 9 mg/kg sc), the urethra and the two ureters were ligated and a polyethylene catheter was positioned into

the bladder to continuously record intravesical pressure. Saline was infused into the bladder to obtain a basal vesical pressure of ~20 cmH₂O. Cumulative doses of drugs (arecoline, phenylephrine, and α,β -MeATP, 3, 10, 30, 100, and 300 μ g/kg iv) were injected in a volume of 1 ml over 1 min every 10 min for arecoline and phenylephrine and every 15 min for α,β -MeATP. Results were expressed as bladder pressure increases (Δ BP, cmH₂O). The dose required to elicit a 10 cmH₂O increase in intravesical pressure (ED₁₀, μ g/kg, intravenous) was calculated by linear regression as it corresponds to an ~50% increase of the basal value.

In Vitro Experiments

Tissue preparation. Two groups of 10- and 30-mo-old female Wistar/Rij rats were specifically used for in vitro studies. The urinary bladder was excised at the level of the bladder base, immediately cleaned from surrounding tissues, and opened longitudinally. Two equal strips were cut from these bladders in a longitudinal direction. The bladder base was isolated, and one circular strip was prepared for each animal. Bladder body and bladder base smooth muscle strips were suspended in 20 ml organ bath containing a modified Krebs solution at 37°C (in mM: 114 NaCl, 4.7 KCl, 1.2 KH₂PO₄, 11.7 glucose, 2.5 CaCl₂, 1.2 MgSO₄·7H₂O, 25 NaHCO₃, and 1.1 ascorbic acid) with 1 μ M propranolol and gassed with a mixture of 95% oxygen and 5% carbon dioxide. One end of each bladder strip was connected to a force displacement transducer (type 351, Hugo Sachs, Germany), and change in muscle tension was measured on a chart recorder (Gould) and on a real-time data-acquisition system (Software Jad, Notocord, France). A basal tension of 0.7 g for the bladder body and 0.5 g for the bladder base was applied to each strip that was washed every 15 min during 1 h equilibration time.

Contractile and relaxant responses in bladder body and bladder base. After the equilibration period, 80 mM KCl was added in the organ baths to measure the maximum contractile response of the strip. Then, the preparations were rinsed several times and allowed to equilibrate for 60 min before performing a cumulative concentration response curve (CRC) to noradrenaline (0.01–100 μ M). Muscle strips were rinsed, after obtaining a plateau of response, to recover their basal tension. After a further 60 min of equilibration time, CRC for carbachol or arecoline, in the range 0.01–30 (or 100) μ M, was

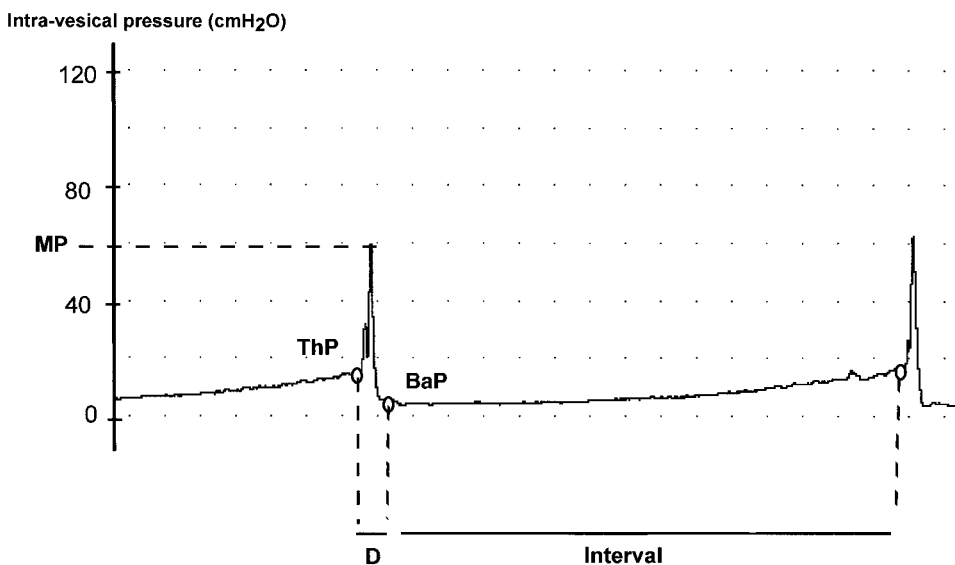


Fig. 1. Cystomanometric parameters investigated in conscious female rats. Basal pressure (BaP, cmH₂O), peak micturition pressure (MP, cmH₂O), threshold pressure (pressure at which micturition occurs; ThP, cmH₂O), micturition duration (D, s), interval (time between 2 subsequent micturitions, min). Five reproducible micturition cycles were analyzed, and means of different cystomanometric parameters were calculated.

obtained in the bladder body, whereas a CRC for phenylephrine (0.1–1,000 μM) was done on the bladder base.

In separate experiments on the bladder body, cumulative CRCs for phenylephrine (0.03–1,000 μM) and for the selective α_2 -adrenoceptor agonist UK 14,304 (0.1–100 μM) were obtained after the CRC for noradrenaline, as described above.

In another series of detrusor muscle strips, α, β -MeATP (0.1–300 μM) was tested in a noncumulative manner to avoid receptor desensitization. Each agonist concentration was added every 25 min with washouts of tissue strips every 5 min. Contractile responses to each agonist tested were expressed as a percentage of the contractile response to 80 mM KCl.

In another set of experiments, the relaxant effect induced by β -adrenoceptor activation was tested. As previously described (29), the detrusor muscle was contracted with 50 mM KCl, rinsed, and equilibrated for 30 min, then again contracted with 50 mM KCl, and, on the plateau of contraction, isoprenaline was added in a cumulative manner in the range 0.001–10 μM . Each concentration was added when a plateau of relaxation was reached. Results are expressed as percentage of the plateau of contraction obtained by the second addition of 50 mM KCl. For this experiment, propranolol was not added in the Krebs solution.

For all these experiments, agonist potencies expressed as pD_2 values ($-\log \text{EC}_{50}$) and maximum responses (E_{max}) were calculated by a regression analysis of the full curves using the ALLFIT software.

Histology and Morphometry

For histology and morphometry, the urinary bladders were removed from 12 rats in each group. They were fixed in 10% Formalin and embedded in paraffin. Transverse histological sections were performed at three levels and were stained with hematoxylin-eosin and orcein elastic stain for qualitative assessment and with Sirius red for morphometric measurement of collagen (35).

Morphometric measurement was based on computerized image analysis using a Nacet NS15,000 processor (Nacet, Evry, France) driven by a microcomputer following a program written in the INSERM Unit (35). For each urinary bladder, three microscopic fields, each in three different transversal sections, were observed through a Nacet microscope and a video camera (Sony, Tokyo, Japan) with a $\times 4$ objective lens, yielding a final calibration of 3.5 $\mu\text{m}/\text{pixel}$. For collagen measurement, the Sirius red-stained sections were observed using polarized light (26). Collagen density in the muscularis and in lamina propria layers as well as mean thickness of the muscularis layer were measured.

Statistical Analysis

The results are given as mean values \pm SE in each group of rats. Unpaired Student's test was used for *in vivo* studies. For morphometric analysis, the comparison of the measurements (the mean of the 9 measurements/rat) between groups was performed using analysis of variance (Statview program, Abacus, Berkeley, CA) with age as a factor. Statistical significance was accepted for $P < 0.05$.

RESULTS

Body Weight and Bladder Weight

The average body weight of 30-mo-old rats (248 ± 5 g; $n = 14$) was significantly ($P < 0.05$) greater than that of 10-mo-old rats (221 ± 5 g; $n = 13$). Similarly, a significant ($P < 0.05$) increase in bladder weight was

observed in senescent rats (0.151 ± 0.007 g) compared with young adult rats (0.133 ± 0.008 g). However, when the bladder weight was expressed as a percentage of body mass, no difference was observed between groups ($0.060 \pm 0.003\%$ and $0.061 \pm 0.002\%$, respectively).

Cystomanometry in Conscious Animals

Five reproducible micturition cycles were analyzed for each rat of both groups, thereby determining individual micturition patterns.

In 30-mo-old rats, we were able to discriminate between three types of micturition patterns: type I (20% of the animals) was characterized by a higher MP without bladder instability; type II, (60% of the animals) by the presence of bladder instability; and type III, by unstable contractions associated with urinary leakage without micturition. In 10-mo-old rats, however, type I (but with MP values significantly smaller) was present in 75% of animals and type II in 25% of the animals only. Typical micturitions patterns are shown in Fig. 2.

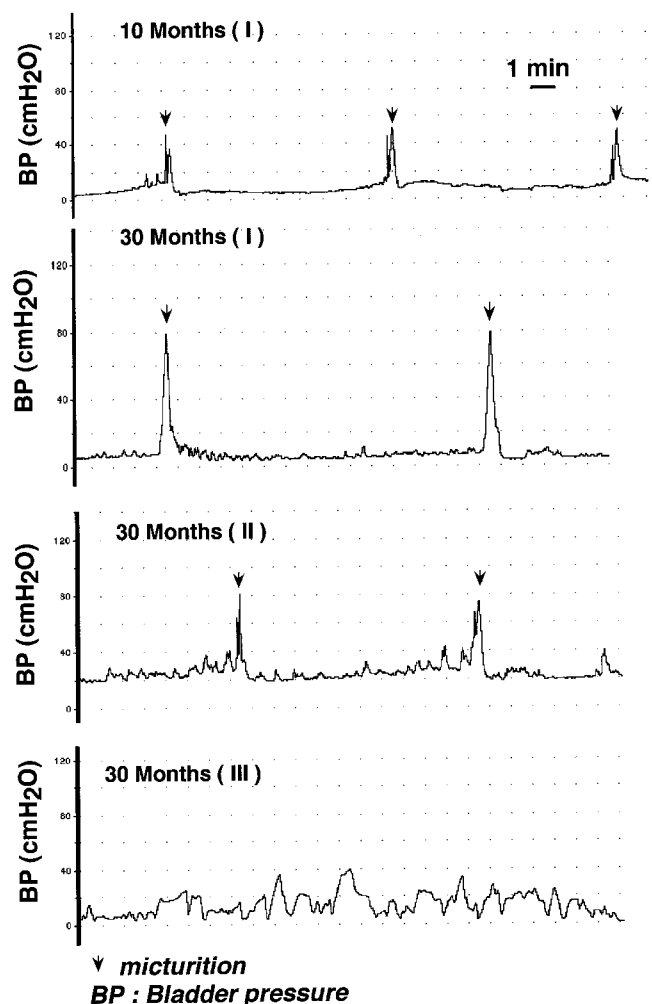


Fig. 2. Different types of micturition profiles obtained in conscious 10- and 30-mo-old female rats. Type I: no instabilities; type II: instabilities mainly before micturition; type III: no micturition, dribbling. BP, bladder pressure.

Table 1. *Cystomanometric parameters in conscious 10- and 30-mo-old female rats*

	10 mo (n = 13)	30 mo (n = 14)
BaP, cmH ₂ O	12 ± 3	15 ± 3
ThP, cmH ₂ O	13 ± 2	13 ± 1
MP, cmH ₂ O	46 ± 3	63 ± 6*
D, s	8 ± 1	12 ± 2*
Interval, min	3.2 ± 0.5	3.6 ± 0.3
MV, ml	0.19 ± 0.02	0.17 ± 0.03
RV, ml	0.13 ± 0.04	0.12 ± 0.02

Values are means ± SE. Basal pressure (BaP, cmH₂O), peak micturition pressure (MP, cmH₂O), threshold pressure (ThP, pressure at which micturition occurs, cmH₂O), micturition duration (D, s), interval (time between 2 subsequent micturitions, min), micturition volume (MV, ml), residual volume (RV, volume infused minus MV, ml). Five reproducible micturition cycles were analyzed, and means of the different cystometric parameters were calculated. *Statistically different from 10 mo, unpaired *t*-test, *P* < 0.05.

Mean values of cystomanometric parameters obtained in conscious animals are shown in Table 1. It is important to note that aging rats showing the type III pattern (Fig. 2) were not included in the cystomanometric analysis. In aging rats (type I and II), MPs and D were significantly higher by ~40–50%. In contrast, bladder capacity, as reflected by interval between mictu-

rition, micturition volume, and residual volume were not modified.

Bladder Contractility in Anesthetized Animals

All agonists tested dose-dependently induced bladder contractions (Fig. 3). The response to arecoline was significantly reduced in the senescent group (ED₁₀ = 36 ± 10 and 129 ± 45 μg/kg in the 10- and 30-mo-old group, respectively, *P* < 0.05). In contrast, the bladder response to phenylephrine was significantly greater in older rats (ED₁₀ = 31 ± 4 and 14 ± 1 μg/kg in the 10- and 30-mo-old group, respectively, *P* < 0.05). The response to α,β-MeATP was not modified with age (ED₁₀ = 29 ± 18 and 13 ± 2 μg/kg in the 10- and 30-mo-old group, respectively, *P* > 0.05).

Responses in the Bladder Body

The intrinsic contractility of the bladder body was unaffected by age because no difference was found in the magnitude of the contraction induced by 80 mM KCl between 10- and 30-mo-old rats (1.94 ± 0.12 and 1.79 ± 0.13 g, respectively, *n* = 8 for each). Similarly, the contractile responses to carbachol, arecoline, and α,β-MeATP were similar in tissues from young adult and senescent rats (Fig. 4, A-C, respectively; Table 2).

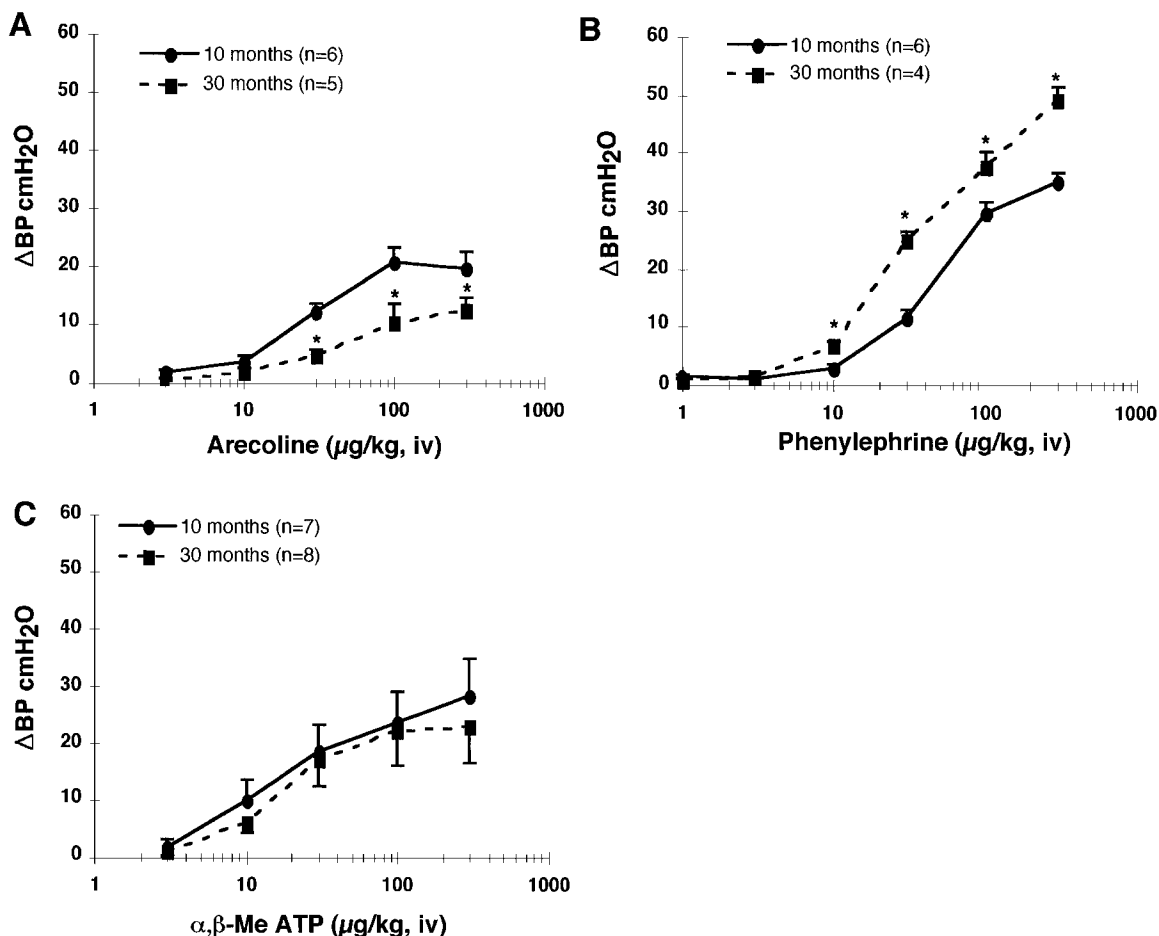


Fig. 3. Bladder pressure increases (ΔBP, cmH₂O) induced by arecoline (A), phenylephrine (B), and α,β-Me ATP (C) in anesthetized female Wistar/Rij rats. *Statistically different from 10-mo-old rats; unpaired *t*-test, *P* < 0.05.

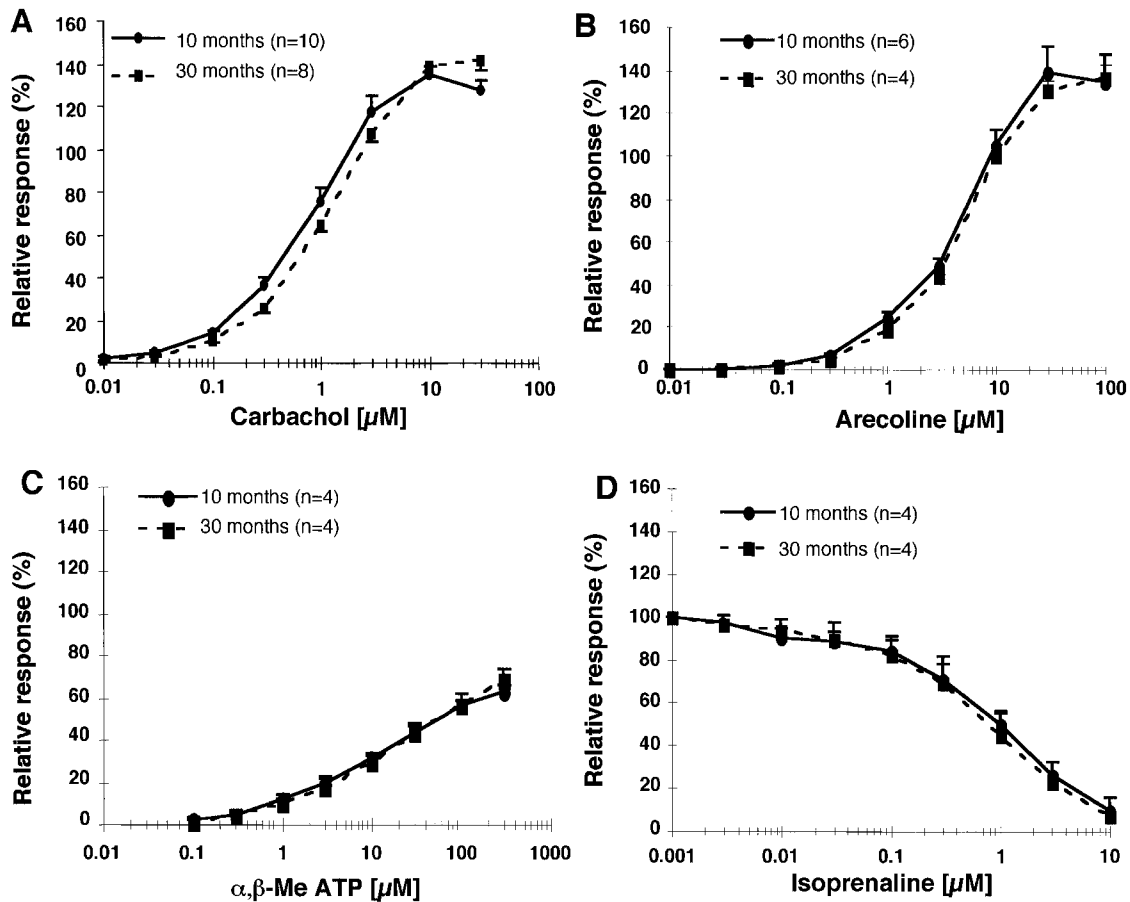


Fig. 4. Concentration-response curves for carbachol (A), arecoline (B), α,β -Me ATP (C), and isoprenaline (D) from 10- and 30-month-old rat isolated detrusor muscle. Responses are expressed as percentage of 80 mM KCl-induced contractions (A-C) or 50 mM KCl (D).

Isoprenaline exhibited the same potencies in relaxing KCl-precontracted bladder body from 10- and 30-month-old rats; pD_2 values being equal to 6.22 ± 0.13 and 6.25 ± 0.19 , respectively (Fig. 4D; $n = 4$ for each).

On the other hand, the maximum contractile responses to noradrenaline were two times greater ($P < 0.01$) in senescent rats, whereas contractile responses to phenylephrine were not modified by aging

(Fig. 5, Table 2). No difference was observed in contractile responses to noradrenaline and phenylephrine in the same age group. Importantly, the selective α_2 -adrenoceptor agonist UK 14,304, in the range 0.01–100 μ M, did not induce any contraction in either group of rats (Table 2; $n = 4$ for each).

Responses in the Bladder Base

No difference was noted in the contractile response to noradrenaline or phenylephrine in the bladder base between 10 and 30 mo (Fig. 5), both in terms of pD_2 or E_{max} responses. For noradrenaline, pD_2 and E_{max} values were 5.62 ± 0.07 and $49.7 \pm 5.6\%$ for 10-month-old rats ($n = 5$), respectively, and 5.56 ± 0.08 and $49.9 \pm 4.4\%$ for 30-month-old rats ($n = 5$), respectively. For phenylephrine, pD_2 and E_{max} values were 5.32 ± 0.12 and $54.1 \pm 4.9\%$ for 10-month-old rats ($n = 5$), respectively, and 5.30 ± 0.18 and $49.9 \pm 4.4\%$ for 30-month-old rats ($n = 5$), respectively.

Histology and Morphometry

Qualitative analysis of the hematoxylin-eosin and elastic stained sections did not show any difference between the two groups. A tiny elastic network in the

Table 2. pD_2 and E_{max} values obtained in 10- and 30-month-old rats isolated bladder body

Agonists	10 mo		30 mo	
	pD_2	E_{max} (% KCl 80 mM)	pD_2	E_{max} (% KCl 80 mM)
Carbachol	6.00 ± 0.05	134.5 ± 4.1	5.85 ± 0.05	141.8 ± 4.9
Arecoline	5.25 ± 0.02	138.3 ± 12.5	5.21 ± 0.04	136.4 ± 6.5
α,β -MeATP	4.94 ± 0.12	62.2 ± 3.9	4.86 ± 0.13	68.1 ± 5.8
Noradrenaline	5.40 ± 0.06	28.4 ± 6.3	5.39 ± 0.07	$53.6 \pm 4.1^*$
Phenylephrine	4.18 ± 0.05	38.5 ± 3.5	4.34 ± 0.07	45.3 ± 4.1
UK 14,304	<4	0	<4	0

Values are means \pm SE of 4 or 5 experiments for all compounds except for carbachol ($n=6-10$). pD_2 , $-\log EC_{50}$; E_{max} , maximum response. Responses obtained for α,β -MeATP and phenylephrine were at the maximal concentration tested. *Statistically different from 10 mo, unpaired t -test, $P < 0.05$.

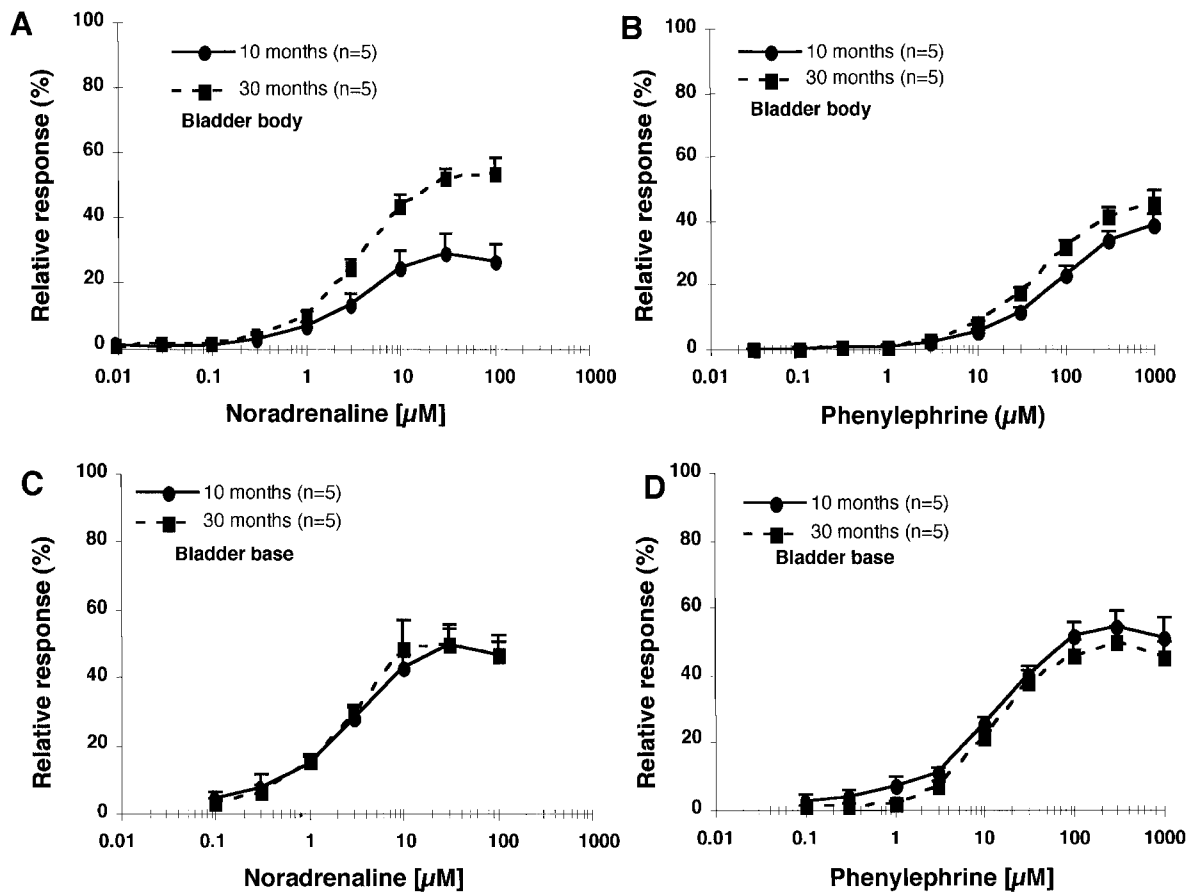


Fig. 5. Concentration-response curves for noradrenaline (A and C) and phenylephrine (B and D) from 10- and 30-mo-old rat isolated bladder base and bladder body. Responses are expressed as percentage of 80 mM KCl-induced contractions.

urinary bladder wall was present in both groups, and no inflammation was observed.

Morphometric analysis showed (Fig. 6) an increase in the mean thickness of the muscularis layer with age: $799.6 \pm 12.9 \mu\text{m}$ in the 10-mo-old rats vs. $1,011.3 \pm 18.2 \mu\text{m}$ in the 30-mo-old rats ($P < 0.0001$, $n = 12$ for each group). The collagen density decreased with age: $16 \pm 1.1\%$ in the 10-mo-old rats vs. $9.4 \pm 0.7\%$ ($P < 0.0001$) in the 30-mo-old rats in the muscularis layer and $27.7 \pm 2.2\%$ in the 10-mo-old rats vs. $12.9 \pm 1.5\%$ ($P < 0.0001$) in the 30-mo-old rats in the lamina propria layer.

DISCUSSION

The aim of the present study was to investigate the effect of normal aging on bladder function in female rats. For this purpose, we have used a strain of female Wistar rat (WAG/Rij) obtained from a specific pathogen-free husbandry. The low incidence of bladder cancers in this strain of rats is in sharp contrast to the high incidence of spontaneously occurring cancers in most other rat strains (2, 14). Moreover, all the female rats used were nulliparous because it is known that

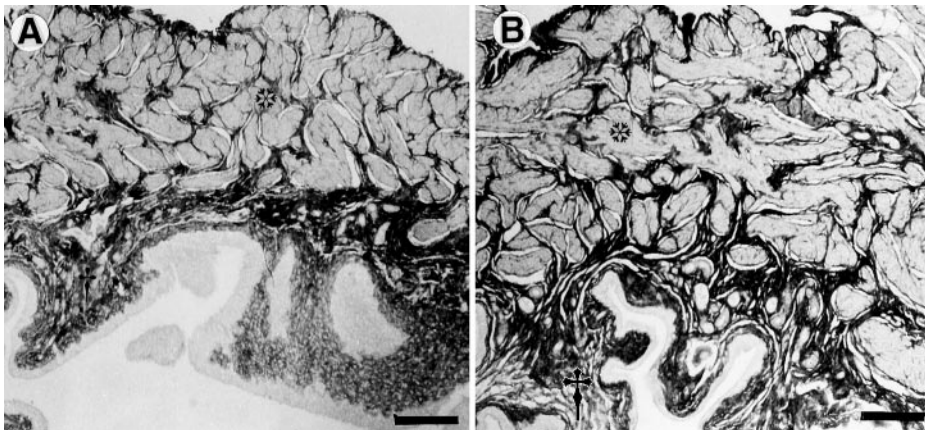


Fig. 6. Transverse histological bladder section from 10 (A)- and 30-mo-old rat (B; Sirius red stained; bar = 200 μm). * Muscularis layer, † lamina propria layer.

parity and/or parturition may induce bladder dysfunctions (11).

Modification of Cystomanometric Parameters

The most important result of our investigation is the observation that 60% of aging rats (but only 25% of 10-mo-old rats) showed a pronounced spontaneous bladder activity during the filling phase (bladder instability). Moreover, an increase of the peak micturition pressure and a longer duration of micturition were noticed in senescent rats. In these rats, we were able to discriminate between three types of micturition patterns. These altered micturition profiles appeared to have common features with those we have reported in conscious female rats following bladder outlet obstruction (23), in which ~80% of the rats presented a detrusor instability and a high MP. In contrast, no modification of the pressure at which micturition occurs (ThP), BaP, bladder capacity, and bladder compliance were observed in our study. These results vary from those previously reported in anesthetized rats, in which an increased ThP for micturition in older animals was demonstrated in the absence of modification in bladder capacity (5, 6). Another study on anesthetized male Wistar rats (16) concluded that aging was associated with a large increase in bladder capacity and a lower MP, which is the opposite of our findings. These contrasting results may be explained by the use of anesthesia that modify detrusor responses (39) or by a difference in strain, gender, and age of animals used. In our study, bladder capacity (as reflected by interval between micturitions) is essentially similar in 10- and 30-mo-old rats. These results are at variance with those of Chun and co-workers (5) but in accordance with another study in WAG/Rij rats, which reported that the frequency of micturition only slightly decreased with age (7). The differences in terms of micturition frequency is probably due to the bladder hypertrophy of the aging rats used by Chun and collaborators (male Fisher 344 rats).

Structural Changes of the Detrusor Muscle

In contrast to rats used by other authors (5, 17), in the present experiments, the increase in bladder weight was associated with a parallel increase in body weight, indicating the absence of vesical hypertrophy in this strain of Wistar rats (Wag/Rij). However, we observed structural changes in the aging bladder. An increase in the mean thickness of the muscularis layer and a decrease in collagen density in the muscularis and in the lamina propria layer was evident in aging bladders. The increase in the quantity of smooth muscle could explain the higher peak micturition pressure observed during cystomanometry in aging rats and may be due to muscle cell hypertrophy, as previously described in the bladder after outlet obstruction (8). The decrease in collagen density could be related to the increase of the mean thickness of the muscularis layer without modification of the total amount of collagen. Aging of the rat urinary bladder seems therefore not associated with

bladder fibrosis, thus explaining the absence of age-related modification of bladder compliance observed in this study. To date, the influence of aging on collagen composition in the rat bladder was not reported, but no difference in collagen content has been shown in the aging mouse bladder (32). It should be noted that in the aging human detrusor muscle, a significant fibrosis does occur (15).

Bladder Contractility in Vitro

We also investigated the contractile response of urinary bladder strips to several agonists. No difference was found in the intrinsic contractility of detrusor muscle between young adult and senescent rats, as indicated by similar responses to 80 mM KCl. Moreover, no age-related differences in the contractile response to the selective P_{2X} -receptor agonist α, β -MeATP were disclosed both in vitro and in vivo. Furthermore, the contractile responses of the isolated detrusor muscle to carbachol and arecoline were similar in the two groups of rats, in accordance with a previous report (27), and it is interesting to note that acetylcholine contractile potency on human detrusor muscle is similar in tissues taken from normal subjects or from patients with detrusor instability (19). However, we found an age-related decrease in the response to intravenous arecoline in anesthetized rats. One possible explanation for the discrepancy between in vitro and in vivo results could be a differential functional role of M_2 muscarinic receptors that, being activated by arecoline together with M_3 receptors, contract the bladder indirectly by reversing β -adrenoceptor-mediated relaxation (13). Our in vitro, but not in vivo experiments, were performed in the presence of propranolol (a potent β_2 -adrenoceptor antagonist), so a possible explanation for such a decreased contractile response to arecoline in vivo in aging rats could be the lack of interaction between M_2 and β_2 -adrenoceptors, which is functional in 10-mo-old rats. Alternatively, age-related changes in the pre- or postjunctional modulation of arecoline-induced contractility by other neurotransmitters or neuromodulators could be evoked.

In humans, a clear correlation between α -adrenergic contractile response in the isolated detrusor muscle and bladder instability was evident in patients suffering from prostatic obstruction (31), and such a correlation to detrusor instability seems also to exist in rats. Interestingly, in the bladder body, we found a clear increase in noradrenaline maximal response in 30-mo-old rats. However, the sensitivity to noradrenaline was unchanged in the bladder base, a region rich in α_1 -adrenoceptors. The lack of effect of the selective α_2 -adrenoceptor agonist UK 14,304 suggests that noradrenaline acts through contractile α_1 -adrenoceptors only. Damage of sympathetic nerves was previously reported in the urinary tract of aging rats (37). However, denervation supersensitivity is not a likely explanation of our results, because in this case, a leftward shift in the CRC for noradrenaline would be apparent (38). Moreover, an increase in α_1 -adrenoceptor density seems not probable, because in this case, the E_{max} for

phenylephrine would be higher also. A possible way to explain our results is to hypothesize a shift in β -adrenoceptor density with age. Several recent papers have demonstrated that noradrenaline-induced relaxation in male rat detrusor is mediated by both β_2 - and β_3 -adrenoceptors (29, 40). In our hands, the relaxant potency of isoprenaline was similar in 10- and 30-month old rats; however, this compound does not discriminate between β -adrenoceptor subtypes, whereas propranolol is 100 times more potent on β_2 - than β_3 -adrenoceptors (29). Therefore, in our in vitro conditions (1 μ M propranolol), β_3 -adrenoceptors are only partially blocked on the detrusor muscle. So, if we hypothesize that in senescent rats, noradrenaline mainly acts on β_2 -adrenoceptors, whereas in young adult rats, it activates β_3 -adrenoceptors, the net result would be an increase in the response to noradrenaline in older animals, as we observed. Because phenylephrine does not activate β -adrenoceptors, this hypothesis explains why phenylephrine responses were similar in the two groups of rats. Interestingly, a previous investigation on Fisher male rats showed no age-related changes in phenylephrine-induced contraction and isoprenaline-induced relaxation on isolated detrusor muscle (20).

In anesthetized rats, phenylephrine induced large bladder contractions, in sharp contrast to the relatively weak contractile effect displayed on the isolated detrusor muscle (~40% of KCl-induced response). It is, therefore, likely that the in vivo response to this adrenergic agonist is not only due to activation of α_1 -adrenoceptors on detrusor muscle itself, but probably involves receptors located on cell bodies of sympathetic ganglia that, in rats, are found in plexuses near the bladder base (1). Therefore, the increase in the response to phenylephrine in senescent rats could be due to a modification in sympathetic tonus at the level of pelvic ganglia.

In conclusion, in aging rats, we observed bladder instabilities during the filling phase and higher MP associated with an increase in the urinary contractility to phenylephrine in vivo and to noradrenaline on the isolated detrusor muscle. Moreover, aged rats demonstrated a decrease in the response to arecoline in vivo but no modification in the response to a P_{2x} -receptor agonist. Histological studies showed an increase in the mean thickness of the muscularis layer with age and a decrease in the collagen density in the muscularis and in the lamina propria layers. In contrast to other tissues, age-related changes in the bladder are not associated with fibrosis and modification of the compliance. The fact that the majority of senescent rats displayed bladder instability suggests that this strain of rat could be a good model for the aged human. However, other characteristics of the aging human urinary tract (urinary frequency, decreased cystometric capacity, and decreased detrusor contractility associated with fibrosis) are not present in this strain of rats.

Perspectives

These experiments show greater changes in the voiding pattern in female senescent rats associated

with changes in the contractile response of the detrusor muscle. We propose that aging modifies urinary bladder function, leading to a dysfunction similar to that induced by obstruction. The possibility that aging induces physiological obstruction is further supported by structural changes of the bladder. External factors to the bladder, e.g., urethra, C fiber afferents, and spinal and supraspinal micturition centers, could be considered as possible underlying factors. Further investigations on age-related changes of the urethral function and age-related modifications of β -adrenoceptor subtypes mediating bladder relaxation and β -adrenoceptor/muscarinic receptor interaction will be particularly interesting.

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REFERENCES

1. Alm P and Elmer P. Adrenergic and cholinergic innervation of the rat urinary bladder. *Acta Physiol Scand* 94: 36, 1975.
2. Burek JD. *Pathology of Aging Rats*. Boca Raton, FL: CRC, 1978, p. 101–107.
3. Burnstock G. The past, present and future of purine nucleotides as signalling molecules. *Neuropharmacology* 36: 1127–1139, 1997.
4. Castleden CM, Duffin HM, and Asherm J. Clinical and urodynamic studies in 100 elderly incontinent patient. *BMJ* 282: 1103–1105, 1981.
5. Chun AL, Wallace LJ, Gerald MC, Levin RM, and Wein AJ. Effect of age on in vivo urinary bladder function in the rat. *J Urol* 139: 625–627, 1988.
6. Chun AL, Wallace LJ, Gerald MC, Wein AJ, and Levin RM. Effects of age on urinary bladder function in the male rats. *J Urol* 141: 170–173, 1989.
7. Corman B and Michel J-B. Glomerular filtration, renal blood flow, and solute excretion in conscious aging rats. *Am J Physiol Regulatory Integrative Comp Physiol* 253: R555–R560, 1987.
8. Gabella G and Uvelius B. Urinary bladder of rat: fine structure of normal and hypertrophic musculature. *Cell Tissue Res* 262: 67–79, 1990.
9. Gorton E and Stanton S. Urinary incontinence in elderly women. *Eur Urol* 33: 241–247, 1998.
10. Gosling JA. Modification of bladder structure in response to outflow obstruction and aging. *Eur Urol* 32, Suppl: 9–14, 1997.
11. Grandadam F, Lluel P, Palea S, and Martin DJ. Pharmacological and urodynamic changes in rat urinary bladder function after multiple pregnancies. *Br J Urol* 84: 861–866, 1999.
12. Hald T and Horn T. The human urinary bladder in aging. *Br J Urol* 82, Suppl. 1: 59–64, 1998.
13. Hedge SS, Choppin A, Bonhaus D, Briaud S, Loeb M, Moy TM, Loury D, and Eglen RM. Functional role of M_2 and M_3 muscarinic receptors in the urinary bladder of rats in vitro and in vivo. *Br J Pharmacol* 120: 1409–1418, 1997.
14. Hicks RM, Wakefield JJ, Vlasov NN, and Pliss GB. *Tumours of the Bladder in Pathology of Tumours in Laboratory Animals*. Geneva: IARC Scientific, vol. 1, part 2, 1976, p. 103.
15. Holm NR, Horn T, and Hald T. Detrusor in ageing and obstruction. *Scand J Urol Nephrol* 29: 45–49, 1995.
16. Hotta H, Morisson JFB, Sato A, and Uchida S. The effects of aging on the rat bladder and its innervation. *Jap J Physiol* 45: 823–836, 1995.
17. Italiano G, Calabro' A, Artibani W, Cisternino A, Oliva G, and Pagano F. Bladder function in the aged rat: a functional and morphological study. *Eur Urol* 27: 232–235, 1995.

18. **Johnson JM, Skau KA, Gerald MC, and Wallace LJ.** Regional noradrenergic and cholinergic neurochemistry in the rat urinary bladder: effects of age. *J Urol* 139: 611–615, 1988.
19. **Kinder RB and Mundy AR.** Pathophysiology of idiopathic detrusor instability and detrusor hyper-reflexia. *Br J Urol* 60: 509–515, 1987.
20. **Kolta MG, Wallace LJ, and Gerald MC.** Age-related changes in sensitivity of rat urinary bladder to autonomic agents. *Mech Ageing Dev* 27: 183–188, 1984.
21. **Lepor H, Sunaryade I, Hartanto V, and Shapiro E.** Quantitative morphology of the adult human bladder. *J Urol* 148: 414–417, 1992.
22. **Lieu PK, Sa'adu A, Orugun EO, and Malone-Lee JG.** The influence of age on isometric and isotonic rat detrusor contractions. *J Gerontol* 52A: M94–M96, 1997.
23. **Lluel P, Duquenne C, and Martin D.** Experimental bladder instability following bladder outlet obstruction in the female rat. *J Urol* 160: 2253–2257, 1998.
24. **Longhurst PA, Eika B, Leggett RE, and Levin RM.** Comparison of urinary bladder function in 6 and 24 month male and female rats. *J Urol* 148: 1615–1620, 1992.
25. **Munro DD and Wendt IR.** Contractile and metabolic properties of longitudinal smooth muscle from rat urinary bladder and the effects of aging. *J Urol* 150: 529–536, 1993.
26. **Nicoletti A, Heudes D, Hinglais N, Appay MD, Philippe M, Sassy-Prigent C, Bariety J, and Michel J-B.** Left ventricular fibrosis in renovascular hypertensive rats. Effects of losartan and spironolactone. *Hypertension* 26: 101–111, 1995.
27. **Ordway GA, Esbenshade TA, Kolta MG, Gerald MC, and Wallace LJ.** Effect of age on cholinergic muscarinic responsiveness and receptors in the rat urinary bladder. *J Urol* 136: 492–496, 1986a.
28. **Ordway GA, Kolta MG, Gerald MC, and Wallace LJ.** Age-related change in α -adrenergic responsiveness of the urinary bladder of the rat is regionally specific. *Neuropharmacology* 25: 1335–1340, 1986b.
29. **Oshita M, Hiraoka Y, and Watanabe Y.** Characterization of β -adrenoceptors in urinary bladder: comparison between rat and rabbit. *Br J Pharmacol* 122: 1720–1724, 1997.
30. **Ouslander JG, Uman GC, Urman HN, and Rubenstein LZ.** Incontinence among nursing home patients: Clinical and functional correlates. *J Am Geriatr Soc* 35: 324–330, 1987.
31. **Perlberg S and Caine M.** Adrenergic response of bladder muscle in prostatic obstruction. *Investig Urol (Berl)* 20: 524–527, 1982.
32. **Phillips JI and Davies I.** The comparative morphology of the bladder and urethra in young and old female C57BL/1crfa^t mice. *Exp Gerontol* 15: 551–562, 1980.
33. **Resnick NM, Yalla SV, and Laurino E.** The pathophysiology of urinary incontinence among institutionalized elderly persons. *N Engl J Med* 320: 1–7, 1989.
34. **Saito M, Kondo A, Gotoh M, Kato K, and Levi RM.** Age-related changes in the response of the rat urinary bladder to neurotransmitters. *Neurourolog Urodyn* 12: 191–200, 1993.
35. **Veniant M, Clozel JP, Heudes D, Banken L, and Menard J.** Effects of Ro 40–5967, a new calcium antagonist, and enalapril on cardiac remodeling in renal hypertensive rats. *J Cardiovasc Pharmacol* 21: 544–551, 1993.
36. **Wagg A and Malone-Lee J.** The management of urinary incontinence in the elderly. *Br J Urol* 82: 11–17, 1998.
37. **Warburton AL and Santer RM.** Sympathetic and sensory innervation of the urinary tract in young adult and aged rats: a semi quantitative histochemical and immunohistochemical study. *Histochem J* 26: 127–133, 1994.
38. **Westfall DP.** Supersensitivity of smooth muscle. In: *Smooth Muscle: An Assessment of Current Knowledge*, edited by Bulbring E, Brading AF, Jones AW, and Tomita T. London, UK: Arnold, 1981, chapt. 13, p. 285–309.
39. **Yaksh TL, Durant PAC, and Brent CR.** Micturition in rats: a chronic model for study of bladder function and effects of anesthetics. *Am J Physiol Regulatory Integrative Comp Physiol* 251: R1177–R1185, 1986.
40. **Yamazaki Y, Takeda H, Akahane M, Igawa Y, Nishizawa O, and Ajisawa Y.** Species differences in the distribution of β -adrenoceptor subtypes in bladder smooth muscle. *Br J Pharmacol* 124: 593–599, 1998.
41. **Yu H-J, Wein AJ, and Levin RM.** Contractile responses and calcium mobilization induced by muscarinic agonists in the rat urinary bladder: effects of age. *Gen Pharmacol* 28: 623–628, 1997.