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Age-related changes in urethrovesical coordination in male rats: relationship with bladder instability?

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Lluel, P., V. Deplanne, D. Heudes, P. Bruneval, and S. Palea. Age-related changes in urethrovesical coordination in male rats: relationship with bladder instability? *Am J Physiol Regul Integr Comp Physiol* 284: R1287–R1295, 2003. First published December 27, 2002; 10.1152/ajpregu.00499.2001.—The micturition profile in conscious animals and the urethrovesical coordination in anesthetized conditions were investigated in 6- and 24-mo-old male Sprague-Dawley rats. The *in vitro* pharmacological responses to KCl, electrical field stimulation (EFS), carbachol, phenylephrine, and isoprenaline were determined in the isolated bladder body, the bladder neck, and urethra. A morphometric and immunohistological study has been included. During conscious cystomanometry, 63% of the aging rats but only 25% of the adult rats showed spontaneous contractions during the bladder-filling phase. In conscious aging rats, basal pressure, threshold pressure, and micturition pressure were also significantly increased. In anesthetized aging rats, a decrease in resting urethral pressure at micturition threshold and the occurrence of a significant delay in urethral relaxation during micturition were associated with an increased residual volume. In all isolated tissues, contractile response to KCl was not modified with aging, whereas age-related decreases in maximal responses to carbachol in the bladder body and to phenylephrine and carbachol in the urethra were observed. In the bladder neck only, we found a significant decrease in the amplitude of neurogenic contractions associated with fibrosis but without decrease in nerve density. These experiments show significant modifications in the voiding pattern of aging rats associated with urethral dysfunction and with regionally specific pharmacological and structural changes of the urinary tract. We propose that aging in rats is characterized by an impairment of the urethrovesical coordination, leading to bladder dysfunctions similar to those induced by bladder outlet obstruction.

urinary bladder; bladder neck; urethra

THE FUNCTIONS of the lower urinary tract to store and release urine are dependent on complex neural mechanisms that regulate the activity of the bladder and the various components of the bladder outlet. During the micturition reflex, the nervous system coordinates the muscles of the detrusor, bladder neck, and urethra to promote urine flow.

It is well known that disturbances of bladder function are common in the elderly population. A shared

urodynamic finding in elderly male and female patients with lower urinary tract symptom is detrusor instability (3, 6, 11, 31). Its etiology was mostly attributed to secondary effects consecutive to central neural pathologies (senile dementia, cerebral vascular accident), aging, or bladder outlet obstruction (BOO). In humans, BOO may result from benign prostatic hyperplasia (BPH), urethral stricture disease, congenital anomalies, or alteration in the urethrovesical reflex. The functional changes that develop in response to BOO include detrusor instability, elevated micturition pressures, and the presence of residual volume of urine. Such findings have been documented in several animal species as well as humans using different methods to evaluate bladder function (2, 19, 27, 32, 34).

Because alterations in the coordinated responses of the bladder and the urethra may lead to voiding dysfunction (9), the mechanisms of the urethrovesical coordination are topics of considerable interest (1, 4, 12, 37, 37a). However, despite the extensive use of the rat for aging research, the effects of aging on urethrovesical coordination are still unknown.

These results were at the origin of the present combined functional and pharmacological study on lower urinary tract of aging rats. We used male Sprague-Dawley (SD) rats because this strain was extensively used for study of lower urinary tract, and consequently more data are available (8, 10, 18, 28, 41).

We evaluated the micturition profile in conscious young adult and aging rats and age-associated changes in the urethrovesical coordination. The *in vitro* pharmacological response to various agonists was also performed in the bladder body, the bladder neck, and the urethra. These results were correlated with morphometric and immunohistological studies. We confirmed that aging is associated with several modifications that could be related to a functional obstruction of the bladder.

MATERIALS AND METHODS

Animals

Young adult (6 mo old) and aging (24 mo old) male SD rats (Harlan, France) were used. Animals were fed *ad libitum* and

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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 Sanofi-Synthelabo Research Ethical Committee following the national and international directives. This work fully conforms with the "Guiding Principles for Research Involving Animals and Human Beings" of the American Physiological Society.

In Vivo Experiments

Cystomanometry in conscious animals. Rats were anesthetized with ketamine (Imalgene, Rhône Mérieux, France), 100 mg/kg body wt ip. The abdomen was exposed. A polyethylene catheter (Merck 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 48 h. Cystomanometric investigations were performed in conscious animals 2 days after the bladder catheter implantation in young adult and aging rats, as previously described (20). At the end of the experiment, animals were killed, and bladder and prostate were removed and weighed.

Cystomanometry in anesthetized animals. All surgical and urodynamic procedures were performed under urethane anesthesia (1 g/kg ip; Sigma Aldrich). Through a midline abdominal incision, the bladder was exposed, a catheter was introduced in the bladder dome to record bladder pressure, and a second was positioned in the proximal urethra via the bladder neck to record intraurethral pressure as previously described (25).

Cystomanometric recordings. The different catheters were connected via a T-tube to a strain gauge and an injection pump (Harvard apparatus 22). The conscious rats were held under partial restraint, whereas anesthetized animals were placed in a supine position. A vial was used to collect urine, and the volume expelled was measured. Warmed saline (37°C) was infused at a rate of 6 ml/h into the bladder for both studies. The urethra was not perfused. Bladder pressure alone or bladder pressure and intraurethral pressure in con-

Table 1. Cystomanometric parameters obtained from conscious 6- and 24-mo-old rats

| | 6 mo (n = 8) | 24 mo (n = 8) |
|-------------------------|--------------|---------------|
| BaP, cmH ₂ O | 15 ± 2 | 23 ± 3* |
| ThP, cmH ₂ O | 22 ± 2 | 28 ± 2* |
| MP, cmH ₂ O | 48 ± 3 | 61 ± 3* |
| D, s | 26 ± 2 | 39 ± 3* |
| Bladder capacity, ml | 0.58 ± 0.07 | 0.83 ± 0.07* |

Values are means ± SE. Three reproducible micturition cycles were analyzed, and means of the different cystomanometric parameters were calculated. BaP, basal pressure; ThP, threshold pressure (pressure at which micturition occurs); MP, micturition pressure (maximal pressure during voiding); D, micturition duration (duration between ThP and BaP); bladder capacity, interval between 2 subsequent micturitions × bladder perfusion rate. *Statistically different from 6 mo, unpaired *t*-test, *P* < 0.05.

scious and anesthetized conditions, respectively, were 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 different cystomanometric parameters measured or calculated are illustrated in Figs. 1–3.

For each animal, three reproducible micturition cycles were analyzed, and the mean of the different cystomanometric parameters was calculated. Micturition cycles were considered reproducible when the variation in micturition pressure values and interval between micturitions did not exceed ±10%. Generally, the first cycle was very different from the others and was never considered. In anesthetized rats, the bladder was emptied before each micturition cycle.

Values are expressed as means ± SE.

In Vitro Experiments

Tissue preparation. Rats were killed by cervical dislocation and exsanguination. The whole urinary tract was removed. The whole bladder was dissected into two longitudinal strips including both anterior and posterior sections. A ring of bladder neck was cut at the level of the two ureters, and the proximal urethra was isolated and mounted as a circular ring.

Tissues were suspended between two silk threads in 5-ml organ baths containing modified Krebs solution of the following composition (in mM): 114 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25 NaHCO₃, 11.7 glucose, and 1.1 ascorbic acid. The solution was maintained at 37°C and gassed with 95% O₂-5% CO₂, pH 7.4.

The upper thread was attached to a Grass FT03 isometric transducer, and force was recorded on a Grass model 7D Polygraph and on a real-time data-acquisition system Maclab/8e interface (AD instruments) and dose-response software (v1.1).

Tissues were allowed to equilibrate under a resting tension of 1 g (bladder body) or 0.5 g (bladder neck and urethra) for at least 60 min, during which time the Krebs solution was replaced regularly.

Contractile and relaxant responses in the bladder body, bladder neck, and urethra. CONTRACTILE RESPONSES. After equilibration, a KCl concentration-response curve (CRC) was constructed by cumulative additions of 10-mM concentration increments from 10 to 80 mM. Tissues were then washed, and CRCs to phenylephrine (0.03–300 μM) were performed by cumulative additions of half-log unit concentration increments. In another set of experiments, CRCs to carbachol (0.01–30 μM) were constructed after an initial contraction with KCl (70 mM).

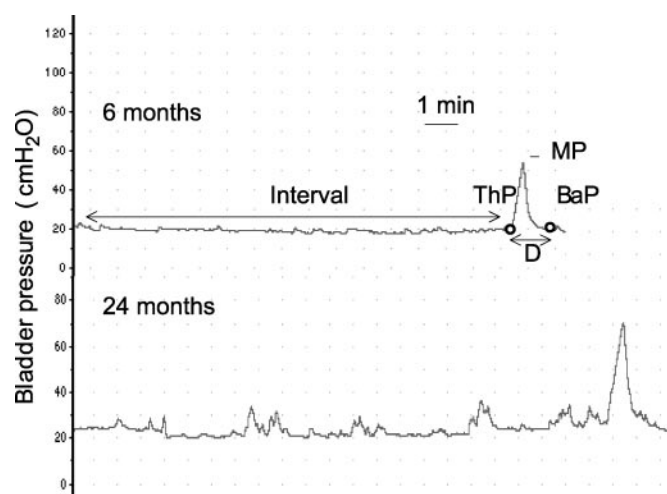


Fig. 1. Typical recording obtained in conscious 6- and 24-mo-old male rats. Circles illustrate points at which the different cystomanometric parameters were calculated: basal pressure (BaP, cmH₂O), peak micturition pressure (MP, cmH₂O), threshold pressure (ThP, pressure at which micturition occurs, cmH₂O), micturition duration (D, s), and interval (time between 2 subsequent micturitions, min; used to calculate bladder capacity = interval × bladder perfusion rate).

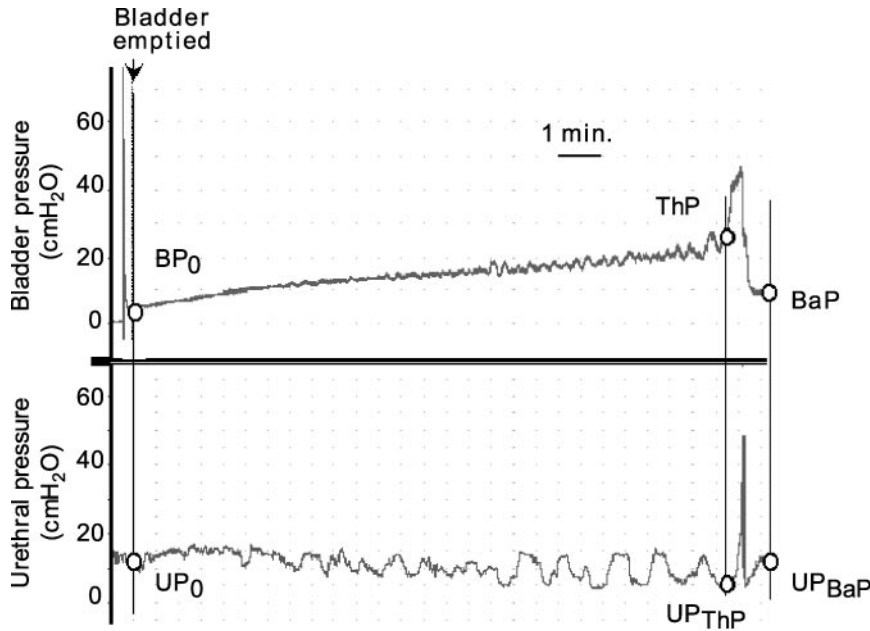


Fig. 2. Typical recording and cystomanometric variables investigated in 6-mo-old anesthetized male rats. Circles illustrate points at which the different cystomanometric parameters were calculated: BP₀ (bladder pressure when bladder is emptied, cmH₂O), ThP (pressure at which micturition occurs, cmH₂O), BaP (cmH₂O), urethral pressure at ThP (UP_{ThP}, cmH₂O), and urethral pressure at BaP (UP_{BaP}, cmH₂O).

In separate experiments, preparations were exposed to electrical field stimulation (EFS; stimulator type 215/I; Hugo Sachs Elektronik) with the following parameters: 50 V, 0.3-ms width, 10-s train width at various frequencies (1–50 Hz) with 1-min interval between each frequency. Preliminary experiments revealed a neurogenic origin of these contractions because TTX (1 μM) completely blocked the responses.

RELAXANT RESPONSES. After a 1-h equilibration period, tissues were contracted by 70 mM KCl, rinsed, and again contracted with 70 mM KCl. On the plateau of contraction, isoprenaline or SR-58611A, a selective β₃-agonist (24), was added in a cumulative manner in the range 1 nM–100 μM.

At the end of in vitro experiments, all the tissues were blotted on a paper and weighed.

Data analysis. All CRCs were fitted by nonlinear regression using the Allfit software to obtain the following parameters: maximal contraction or relaxation induced by the agonist (E_{max}) and agonist concentration that induces 50% of the maximum effect (EC₅₀), expressed as pD₂ (–log EC₅₀).

Table 2. Cystomanometric parameters obtained from anesthetized 6- and 24-mo-old rats

| | 6 mo (n = 8) | 24 mo (n = 8) |
|---|-----------------|------------------|
| <i>Bladder pressure</i> | | |
| BP ₀ , cmH ₂ O | 8 ± 2 | 8 ± 2 |
| ThP, cmH ₂ O | 28 ± 3 | 25 ± 4 |
| MP, cmH ₂ O | 53 ± 4 | 49 ± 2 |
| BaP, cmH ₂ O | 9 ± 1 | 11 ± 1 |
| D, s | 25 ± 3 | 22 ± 1 |
| Bladder capacity, ml | 0.96 ± 0.18 | 0.96 ± 0.17 |
| Bladder compliance, ml/cmH ₂ O | 0.062 ± 0.012 | 0.064 ± 0.080 |
| MV, ml | 0.76 ± 0.16 | 0.39 ± 0.06* |
| RV, ml | 0.21 ± 0.07 | 0.57 ± 0.14* |
| <i>Urethral pressure</i> | | |
| UP ₀ , cmH ₂ O | 14 ± 1 | 14 ± 1 |
| UP _{ThP} , cmH ₂ O | 27 ± 3 | 18 ± 4*† |
| UP _{BaP} , cmH ₂ O | 17 ± 1 | 13 ± 2 |
| Maximal UP, cmH ₂ O | 39 ± 2 | 36 ± 3 |
| Duration of UR, s | 4 ± 1 | 3 ± 1 |
| Amplitude of UR, cmH ₂ O | 27 ± 3 | 31 ± 2 |

Values are means ± SE. Three reproducible micturition cycles were analyzed, and means of the different cystometric parameters were calculated. BP, bladder pressure; UP, urethral pressure. [With the exception of BP₀ (BP bladder emptied, cmH₂O), all vesical parameters are similar to those shown in Fig. 1.] UP₀, UP bladder emptied; UP_{ThP}, UP at micturition (ThP); UP_{BaP}, UP at basal bladder pressure (BaP); maximal UP, maximal UP before urethral relaxation (UR); duration of UR, duration between maximal UP and UP_{BaP}; amplitude of UR, maximal UP – UP_{BaP}. *Statistically different from 6 mo, P < 0.05. †Statistically different from ThP in the same age group, unpaired t-test, P < 0.05.

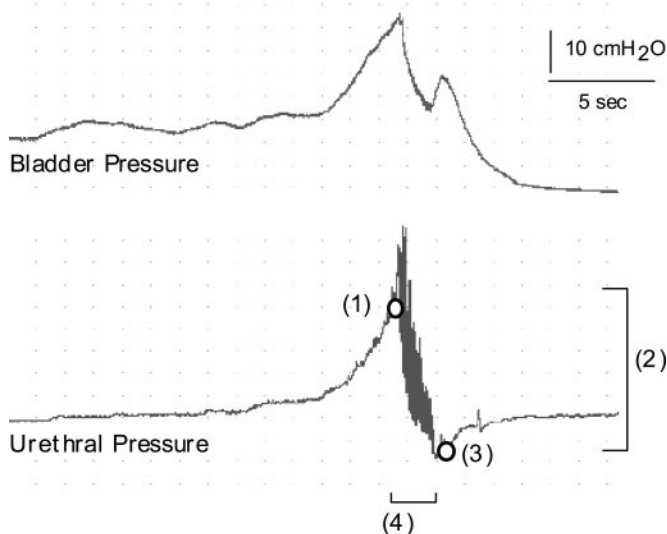
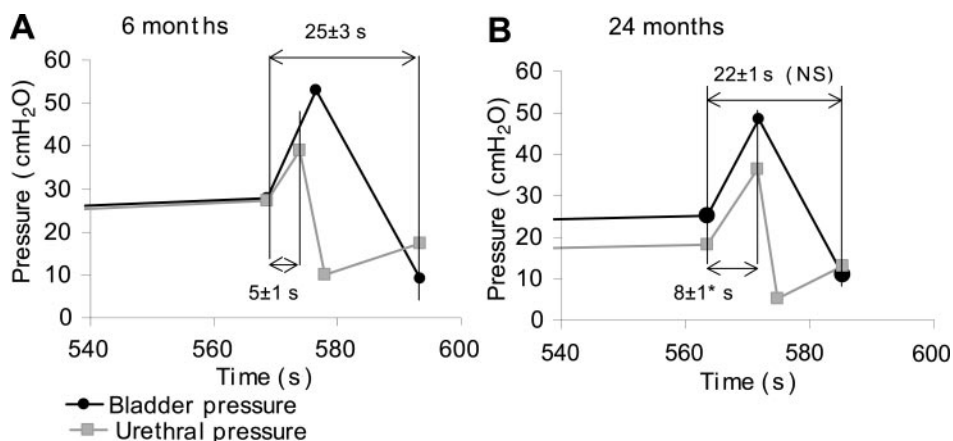


Fig. 3. Typical micturition pattern obtained in 6-mo-old anesthetized male rat. Circles illustrate points at which the different cystomanometric parameters were calculated: maximal UP (1), amplitude of urethral relaxation (2), UP_{BaP} (3), and duration of urethral relaxation (4).

Fig. 4. Mean curves of urethral and bladder pressure variations (average of 8 rats in each group) during micturition in anesthetized 6-mo-old (A) and 24-mo-old male rats (B). Values are taken from Table 2. *Statistically different from 6 mo, unpaired *t*-test, *P* < 0.05.



Effects were given as milligrams of contraction (or relaxation) per milligram of tissue and are expressed as means \pm SE.

Drugs. Phenylephrine hydrochloride, carbachol (carbamylcholine chloride), isoprenaline bitartrate, and KCl were purchased from Sigma (L'Isle D'Abeau Chesnes, France), and TTX was purchased from Research Biochemicals International (Illkirch, France). SR-58611A was synthesized by the Chemistry Department of Sanofi-Synthélabo Recherche. SR-58611A was dissolved in ethanol:distilled water (3:7). All other compounds were dissolved in distilled water.

Histology

The urinary bladders were removed from eight rats in each group. Bladder body and bladder neck were separated as described for *in vitro* experiments. They were fixed for 24 h with 1/10 formalin. For histology, 5- μ m-thick sections were stained with hematoxylin-eosin (for qualitative assessment) and Sirius red (for collagen and bladder wall thickness morphometric analysis).

Morphometric analysis was performed as previously described (20). Briefly, Sirius red-stained sections were observed with a $\times 4$ objective lens (final calibration: 3.5 μ m/pixel) through a microscope and a video camera and measured using a computerized image analysis processor (Nachet NS15,000, Evry, France). The whole circumference of the bladder section was scanned in adjacent fields. The number of fields (mean \pm SE) used for histology was 11.06 \pm 0.69 and 3.0 \pm 0.42 for the bladder body and bladder neck, respectively. The thickness of the bladder wall (including lamina propria and muscularis layers) and the density of collagen were measured.

For immunohistochemistry, all the neuronal processes and nerves were labeled with an anti-human neurofilament protein antibody (Dako, Trappes, France) that cross-reacts with rat tissues, diluted at 1/100. In deparaffinized bladder neck sections, antigen retrieval was achieved before primary antibody incubation with microwave heating three times for 5 min in 2 \times SSC buffer (pH 6.2, 600 mM), and peroxidase

Table 3. pD_2 and E_{max} values obtained in isolated bladder body, bladder neck, and urethra taken from 6- and 24-mo-old rats

| Agonists | 6 mo | | | 24 mo | | |
|---------------------|-----------------|------------------|----------|-----------------|-------------------|----------|
| | pD_2 | E_{max} | <i>n</i> | pD_2 | E_{max} | <i>n</i> |
| <i>Bladder body</i> | | | | | | |
| KCl | | 92.0 \pm 10.4 | 9 | | 81.7 \pm 8.5 | 8 |
| Phenylephrine | 5.22 \pm 0.17 | 7.5 \pm 1.0 | 5 | 5.07 \pm 0.17 | 8.2 \pm 1.3 | 4 |
| Isoprenaline | 5.8 \pm 0.2 | -20 \pm 4 | 9 | 5.7 \pm 0.2 | -23 \pm 8 | 6 |
| SR-58611A | 5.3 \pm 0.3 | -15 \pm 4 | 9 | 5.5 \pm 0.2 | -25 \pm 5 | 6 |
| Carbachol | 5.82 \pm 0.04 | 213.9 \pm 23.3 | 8 | 5.99 \pm 0.05 | 145.2 \pm 22.0* | 8 |
| <i>Bladder neck</i> | | | | | | |
| KCl | | 94.4 \pm 22.7 | 9 | | 84.5 \pm 41.5 | 8 |
| Phenylephrine | 4.85 \pm 0.17 | 31.9 \pm 3.2 | 3 | 5.16 \pm 0.13 | 26.6 \pm 2.3 | 3 |
| Isoprenaline | 6.8 \pm 0.2 | -19 \pm 6 | 4 | 7.1 \pm 0.1 | -19 \pm 3 | 4 |
| SR-58611A | NP | NP | | NP | NP | |
| Carbachol | 5.66 \pm 0.04 | 76.0 \pm 14.3 | 5 | 5.68 \pm 0.08 | 71.5 \pm 13.9 | 5 |
| <i>Urethra</i> | | | | | | |
| KCl | | 102.3 \pm 11.8 | 9 | | 75.5 \pm 17.8 | 9 |
| Phenylephrine | 5.00 \pm 0.12 | 147.4 \pm 14.6 | 5 | 4.81 \pm 0.08 | 81.5 \pm 18.1* | 4 |
| Isoprenaline | 7.3 \pm 0.3 | -37 \pm 5 | 4 | 6.8 \pm 0.2 | -41 \pm 12 | 4 |
| SR-58611A | > 4 | 0 | 4 | > 4 | 0 | 4 |
| Carbachol | 6.05 \pm 0.12 | 95.2 \pm 20.5 | 4 | 6.07 \pm 0.04 | 50.4 \pm 16.0* | 4 |

Values are means \pm SE. $pD_2 = -\log(EC_{50})$; E_{max} , maximum response expressed in mg contraction (or relaxation)/mg of tissue. *Statistically different from 6 mo, Allfit software, *P* < 0.001. NP, not performed.

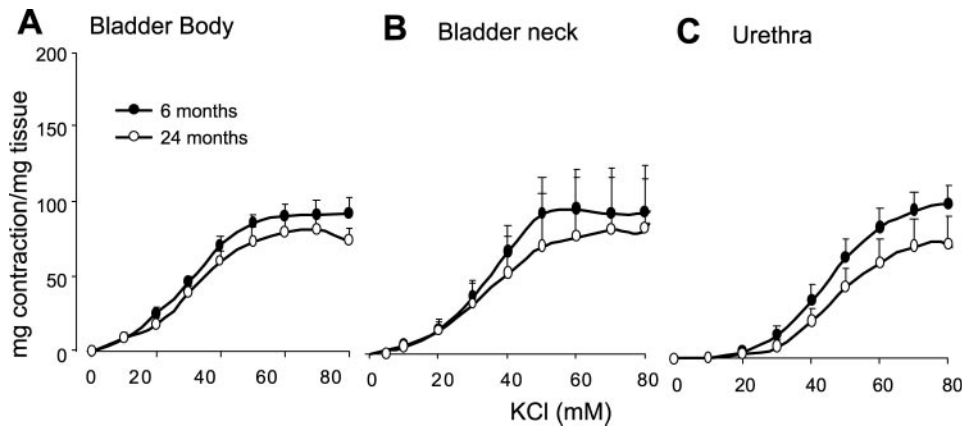


Fig. 5. Concentration-response curves to KCl in isolated bladder body (A), bladder neck (B), and urethra (C) taken from 6- and 24-mo-old rats. Maximal contractile responses were similar in all tissues; $n = 8-9$ rats for each group.

technique was used yielding a dark brown reaction product in neuronal structures. Using an eye-piece grid, the number of neuronal structures per field was measured, as well as the relative area of neuronal structures per field. The mean size of labeled neuronal structures was calculated from the ratio of the relative area to the number. The whole circumference of the bladder section was scanned in adjacent fields with a $\times 10$ objective lens.

Statistical Analysis

For in vitro experiments, agonist potencies expressed as pD_2 values ($-\log EC_{50}$) and E_{max} were compared by a non-linear regression analysis of the full curves using Allfit software. For in vivo studies, unpaired Student's *t*-test was performed using Microsoft Excel software. For histology studies, means per rat of each parameter were calculated and submitted to ANOVA test for age group factor. $P < 0.05$ was accepted for statistical significance.

RESULTS

Bladder, Prostate, and Body Weights

The average body weight of 24-mo-old rats (623 ± 16 g; $n = 8$) was significantly ($P < 0.05$) greater than that of 6-mo-old rats (529 ± 10 g; $n = 8$). Similarly, a significant ($P < 0.05$) increase in bladder weight was observed in aging rats (181 ± 11 mg) compared with young adult rats (131 ± 6 mg). Moreover, when the bladder weight was expressed as a percentage of body mass, this difference remained significant ($0.025 \pm$

0.001 and $0.029 \pm 0.001\%$ for 6- and 24-mo-old rats, respectively; $P < 0.05$). In contrast, no significant changes in prostate weight were observed when expressed in milligrams (850 ± 45 and 850 ± 87 mg for 6- and 24-mo-old rats, respectively; $P > 0.05$) or in percentage of body mass (0.16 ± 0.01 and $0.14 \pm 0.02\%$ for 6- and 24-mo-old rats, respectively; $P > 0.05$).

Cystomanometry in Conscious Animals

Urodynamic values were obtained in eight individuals for each group of 6- and 24-mo-old rats. Three reproducible voiding cycles were analyzed for each rat of both groups, thereby determining individual voiding patterns: 63% of the conscious aging rats showed spontaneous contractions (>8 cmH₂O) during the bladder-filling phase, whereas only 25% of the adult rats showed similar altered micturition patterns. Typical voiding patterns of each group are shown in Fig. 1 (mean values of the cystomanometric variables obtained in conscious animals are shown in Table 1). There was a significant increase (+43%, $P < 0.05$) in bladder capacity in aging rats without significant modification of micturition volume. The peak micturition pressure was also significantly greater in aging rats (+27%, $P < 0.05$) as well as duration of voiding (+50%, $P < 0.05$). In addition, basal pressure as well as threshold pressure were also significantly increased with age (+53 and +27%, respectively, $P < 0.05$) (Table 1).

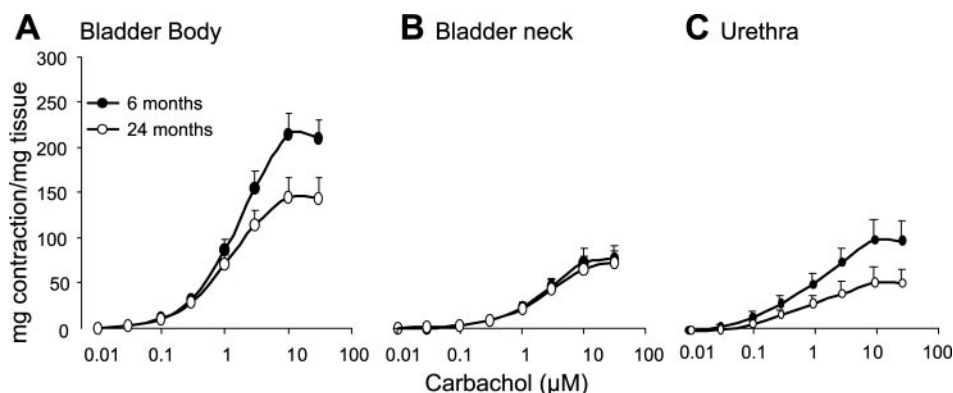


Fig. 6. Concentration-response curves to carbachol in isolated bladder body (A), bladder neck (B), and urethra (C) taken from 6- and 24-mo-old rats. Maximal contractile responses were significantly decreased in bladder body and urethra in aging rats ($P < 0.001$); $n = 4-8$ rats for each group.

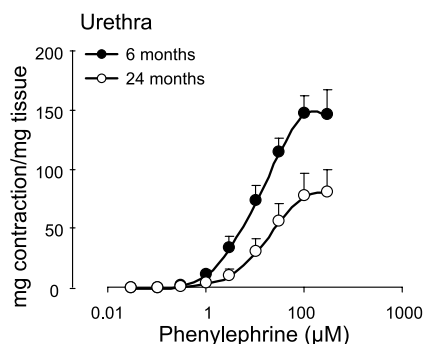


Fig. 7. Concentration-response curves to phenylephrine in isolated urethra taken from 6- and 24-mo-old rats. The maximal contractile response was significantly decreased in aging rats ($P < 0.001$); $n = 4-5$ rats for each group.

Cystomanometry in Anesthetized Rats: Urethrovesical Coordination

Repeated recordings of bladder and urethral pressures were reproducible in both groups.

During filling, urethra and bladder contractions were observed in a similar manner in adult and aging rats (Fig. 2). Interestingly, in some animals of both groups, bladder pressure exceeded urethral pressure without any leakage of urine. At micturition, the bladder pressure increase is associated with a coordinated reduction in urethral pressure (Fig. 3). This urethral relaxation occurred only during micturition contraction and was always associated with emission of urine. Urethral pressure was undergoing several variations during the micturition cycle. First, we observed an increase in urethral pressure in conjunction with an initial rise in bladder pressure followed by a large decrease in urethral pressure during emission of urine. A second component was superimposed on the urethral relaxation and consisted of high-frequency contractions (Fig. 3) as previously described (1). After urethral relaxation, urethral pressure returned to baseline. Qualitatively, no difference was observed between the two age groups for micturition patterns.

To quantify these micturition patterns, bladder and urethral pressures recorded during three micturition cycles were analyzed, and means of different cystomanometric parameters were used to characterize each

animal (Table 2). For both groups, mean curves of urethral pressure and bladder pressure variations during micturition (average of 8 experiments for each group) were shown in Fig. 4.

Quantitative differences appeared with age especially on the urethral relaxation and urethral pressure at micturition. At threshold micturition pressure, urethral pressure was significantly lower (-30% , $P < 0.05$) in aging rats than in adult. In addition, although amplitude and duration of urethral relaxation were similar in both groups (Table 2), urethral relaxation occurred significantly later in aging rats than in adult (with regard to initial bladder contraction, 5 vs. 8 s; $+60\%$, $P < 0.05$), with an unchanged micturition duration. In adult rats, urethral relaxation appeared between threshold micturition pressure and peak micturition pressure, whereas in aging rats, urethral relaxation occurred simultaneously with the peak micturition pressure (Table 2, Fig. 4). In these experimental conditions, bladder compliance, peak micturition pressure, and bladder capacity were not modified with aging. In contrast, aging rats expelled significantly less urine (about -50% , $P < 0.05$), and consequently a significant increase in residual volume was noticed with age (Table 2).

In Vitro Results

In both groups, contractile and relaxant properties of the bladder body, bladder neck, and urethra were evaluated using KCl, specific agonists, and EFS.

Contractile responses to KCl (Fig. 5, Table 3) and relaxant responses to isoprenaline were unchanged with age in all tissues studied (Table 3). In bladder body, relaxant responses to the selective β_3 -agonist SR-58611A were similar in both groups. In the isolated urethra of 6- and 24-mo old rats, no net relaxant effect to SR-58611A was detected when compared with relaxation induced by the solvent. In contrast, the maximal contractile responses to carbachol (E_{max}) were significantly lower in aging ($P < 0.001$) than in adult rats both in bladder body and urethra, although there was no difference in agonist potency (pD_2 ; Table 3, Fig. 6). Similarly, in urethra, maximal responses to phenylephrine were significantly lower in aging than in adult

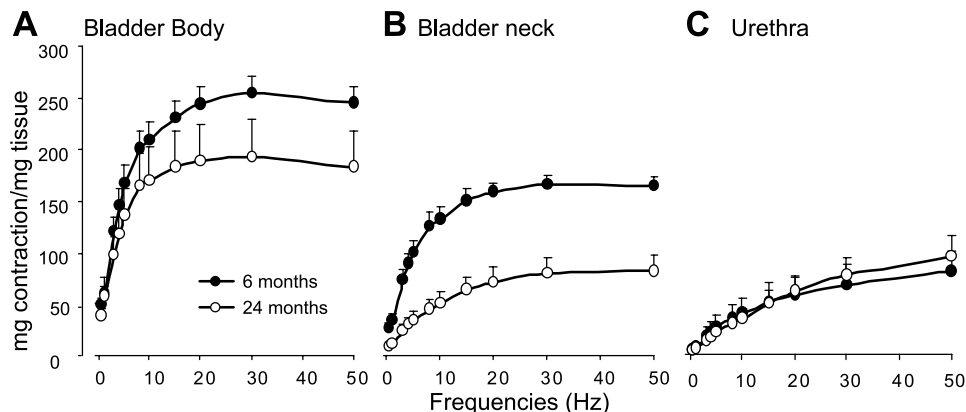


Fig. 8. Concentration-response curves to electrical field stimulation (EFS; 1–50 Hz) in isolated bladder body (A), bladder neck (B), and urethra (C) taken from 6- and 24-mo-old rats. Maximal contractile responses were significantly decreased in bladder neck in aging rats ($P < 0.001$); $n = 4-8$ rats for each group.

Table 4. Morphometric parameters obtained in 6- and 24-mo-old rat urinary bladders

| | 6 mo (n = 6) | 24 mo (n = 8) |
|-------------------------------|-----------------|------------------|
| Wall thickness, μm | | |
| Bladder body | 962 \pm 81 | 978 \pm 53 |
| Bladder neck | 781 \pm 57 | 805 \pm 37 |
| Collagen density, % | | |
| Bladder body | 2.5 \pm 0.2 | 2.8 \pm 0.2 |
| Bladder neck | 1.5 \pm 0.2 | 2.4 \pm 0.1* |
| Nerve density, % | | |
| Bladder body | 4.41 \pm 0.43 | 5.42 \pm 0.40 |
| Bladder neck | 1.37 \pm 0.07 | 1.44 \pm 0.10 |

Values are means \pm SE. *Statistically different from 6 mo, ANOVA test for age group factor, $P < 0.001$.

rats ($P < 0.001$), but its potency was unchanged (Table 3, Fig. 7).

No statistical age-related differences in EFS-induced contractions were evidenced in either bladder body or urethra. In contrast, in the bladder neck, the magnitude of contractions was significantly reduced in aging rats compared with adult rats in the range 3–50 Hz ($P < 0.001$; Fig. 8). These contractile responses were completely blocked by TTX, indicating a neurogenic origin (data not shown).

Histology and Morphometry

Qualitative histological analysis of the hematoxylin-eosin-stained sections did not show any change with age. No sign of inflammation was observed. Morphometric analysis showed no significant change in whole bladder wall thickness with age (Table 4). The density of collagen within the bladder wall was increased with age only at the level of the bladder neck (Table 4). No significant difference was observed in the bladder body.

In the bladder neck, labeled neuronal processes and nerves exhibited no significant change in number and size with aging (Table 4, Fig. 9).

DISCUSSION

The present data show that conscious aging rats developed significant bladder dysfunctions like bladder instabilities during the filling phase and increased values for basal pressure, micturition pressure, and micturition duration. These altered patterns were close to those observed in conscious aging female Wistar rats (20) and rats after BOO (19, 23). In addition, the higher basal pressure suggests a deficit in inhibitory neurotransmission to the detrusor muscle (β -adrenergic or nitrenergic). However, we did not detect changes in relaxant responses of the bladder to $\beta_1/\beta_2/\beta_3$ -adrenoceptor agonists. The higher micturition pressure could suggest an increased contractility of the detrusor smooth muscle. This was not the case because we failed to observe any age-related changes in the contractile responses to EFS and even a decrease in maximal response to carbachol. It should be pointed out that a decrease in muscarinic agonist-induced contractions was also observed in the urinary bladder taken from rat, rabbit, or humans after BOO (16, 33). Interestingly, in the same strain of aged rats, an increased response of detrusor muscle to ATP has been shown (18). Consequently, we believe that the increase in micturition pressure in conscious aging rats could be due to an increased sensitivity to purinoceptor activation.

Age-related changes in cystomanometric parameters observed in conscious rats were not present in anesthetized rats, particularly increased micturition pressure and bladder instabilities. However, this is not surprising because it is well known that anesthesia

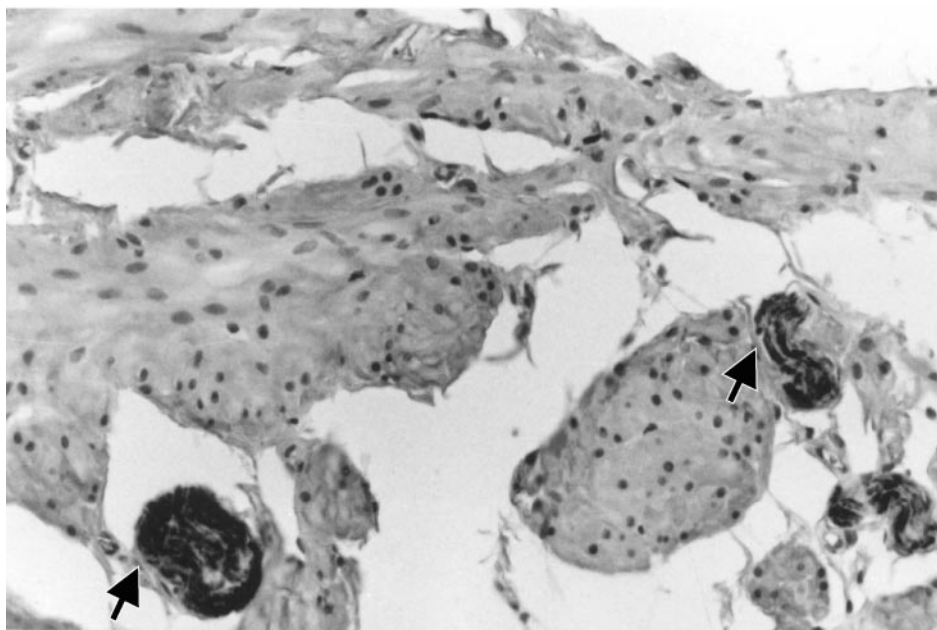


Fig. 9. Immunohistochemical labeling of the nerves in the urinary bladder neck wall in a 24-mo-old rat. Peroxidase technique with an anti-neurofilament protein antibody. Magnification, $\times 400$. Arrows show nerves.

depresses micturition contractions (26) and that urethane inhibits bladder instability (39). In anesthetized aging rats, we found a significant increase in residual volume, which is a characteristic of BOO. Because the bladder was emptied before each micturition cycle, this protocol allows us to measure precisely urinary volume.

Urinary bladder changes in aging rats were also confirmed by a marked increase in bladder weight as reported in the same strain of rats (10, 28, 36, 41). Morphometric analysis did not show any age-related change in the bladder muscle thickness. Fibrosis was specifically observed in bladder neck but not in bladder body in accordance with our findings in aging female Wistar rats (20).

Bladder instability, increased micturition pressure, and residual volume, together with bladder hypertrophy, fibrosis, and a decrease in muscarinic-induced contractions, are associated with aging and are also the consequences of experimental BOO. Relations between aging and BOO have been frequently evoked (17, 21). In humans BOO is essentially related to BPH. However, several symptoms of BOO are also found in female (7) and in spinal cord injury patients (35). The aim of the present study was to understand the mechanisms responsible for the functional obstruction in aging male rats. Three possible mechanisms could be advanced: 1) prostatic enlargement, 2) increased urethral tone, or 3) urethrovesical dysfunctions.

In aging rats, BOO is not due to the prostate because the urethra is not completely encircled by this organ, and furthermore prostatic enlargement was not observed in the present study. Consequently, the first hypothesis was rapidly discarded.

The second and the third hypotheses were evaluated using anesthetized rats and by studying contractility of the bladder outlet components, namely isolated urethral and bladder neck smooth muscles. Intravesical and urethral pressures were recorded simultaneously and independently in anesthetized rats. First, we found a significant decrease in urethral pressure at threshold micturition. Therefore, the functional obstruction is unlikely due to an age-related increase in urethral tone. The most important finding was the occurrence of a significant delay in urethral relaxation during micturition. Consequently, bladder contractions compete against a closed bladder outlet for a longer duration. We speculate that this alteration leads to a functional obstruction. To date, our knowledge of the urethrovesical coordination is quite incomplete (12, 37a), and there is no report on age-related changes. For this reason, we investigated bladder neck and urethral responses using KCl, EFS, and various agonists.

In the rat urethra, the contraction is mediated by both cholinergic and adrenergic nerves (22). In this tissue we found an age-related decrease in the maximal response to phenylephrine and carbachol that could explain the lower urethral pressure observed in aging animals. These decreases were not associated with modification in the global contractile response to

EFS. Therefore, we suggest that an age-related decrease in the inhibitory neurotransmission could compensate the decrease in the contractility of urethral smooth muscle. As we failed to detect age-related changes in β -adrenergic-induced relaxations, we hypothesize a decrease in NO-mediated relaxation. The implication of nitrergic pathways mediating relaxation in urethral smooth muscle is now well established (1, 13, 15, 29, 30).

In the bladder neck, the most important observation is the decrease in EFS-induced contraction in aging rats. To date, it is difficult to explain the relation between this result and the functional obstruction. In any case, this decrease is not due to the fibrosis we observed, because in this tissue the intrinsic contractility was not modified with aging as shown by KCl responses. In addition, no age-related change in the response to α/β -adrenergic and muscarinic agonists was observed. Using immunohistochemistry, no sign of denervation was evidenced in aging rats. We hypothesize that aging of the bladder neck could be associated with an increased relaxation induced by ATP through P2Y purinoceptors, as reported in mini-pig bladder neck (38). Alternatively, a modification in afferent innervation could be suspected. In this context, it is of interest to note that in SD rats, the plexus of afferent axons has been specifically localized in the bladder neck and in the proximal urethra (5).

Perspectives

In summary, these experiments show changes in the voiding pattern of aging rats associated with regionally specific pharmacological and structural changes of urinary tract tissues. We suggest that aging modifies urethrovesical coordination, leading to bladder dysfunctions similar to those induced by BOO. Of particular interest, aging of the lower urinary tract in rats results in an imbalance in neuronal pathways controlling micturition, especially at the level of cholinergic and nonadrenergic-noncholinergic neurotransmissions. Therefore, studies on the implication of purinergic and nitrergic pathways on the contractility of the detrusor and the bladder outlet will be of interest to understand the functional obstruction observed in aging rats.

Moreover, since it has been recently reported (40) that conscious spinal cord-injured rats showed cystomanometric changes similar to those observed with aging in the present study, age-related modifications in spinal micturition pathways could also be suspected as an underlying factor.

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