The Effects of Aging on the Rat Bladder and Its Innervation

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Abstract 1) Measurements of the cystometrogram, of the responsiveness of bladder muscle to pelvic nerve efferent stimulation and of the sensitivity of the pelvic nerve afferents to pressure and volume during distensions have been made in the bladders of young adult (2–3 months) and aged (26–29 months) rats, anesthetized with mixtures of urethane and chloralose. 2) The pressure-volume relationship differed in young adult and aged rats. The bladders of the aged rats held up to nearly six times the volume of the young animals, and these volumes were accommodated at lower pressures in the aged animals. The pressure at which micturition contractions appeared was similar in young adult and aged animals. 3) The passive pressure associated with each of a series of distending volumes was recorded when a pelvic nerve was cut unilaterally. The distal cut end of this cut pelvic nerve was stimulated for 10 s at 20 Hz, using square wave pulses of 10 V and 1.0 ms. The active pressure-volume relationship was constructed from this data. Both the active and the passive relationships were shifted to the right in the aged animals, and it was evident that aging was associated with a reduction in the maximal pressure generated during pelvic nerve stimulation. Also the change in intravesical pressure induced by bladder contraction was less in aged animals. 4) The most sensitive mechanoreceptor afferents appear to have pressure and volume thresholds that do not change significantly during aging. While the distension-sensitive afferents in the pelvic nerve appear to have a similar sensitivity to intravesical pressure in young adult and aged rats, they were less able to monitor volume in the aged animals. The stimulus response relationship for volume was often less steep in the aged animals. 5) In this study, aging was shown to be associated with a large increase in bladder volume and a reduced sensitivity of pelvic nerve afferents to volume, and a reduced

Received on May 30, 1995; Accepted on August 30, 1995

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ability to raise bladder pressure during contraction of bladder smooth muscle. The changes in bladder function associated with aging are discussed.

Key words: aging, bladder, pelvic nerve, cystometry, rat.

A variety of changes in bladder function have been described during aging in humans and other species. Clinically, the frequency of micturition and the voided volume of urine are known to be increased, and there is a reduced ability to suppress the sensation of bladder fullness once it has been noted. These signs and symptoms are accompanied by urodynamic changes including an increased residual volume and a reduction in the maximum squeeze pressure generated by the urethra [1]. In animal experiments, an increase in water intake and urine output has been noted in old rats, and the volume voided and frequency also increased [2]. It is also suggested that the volume of the bladder was similar in young and old rats, but that micturition occurred at higher pressures in the older animals [2, 3], an observation that was not confirmed in the present investigation.

Other experimenters have noted that the responsiveness of vesical smooth muscle to neurotransmitters and other chemicals may or may not change in old age. Saito et al. [4] found that there was no significant age-related difference in the response to acetylcholine, prostaglandin F_2α, angiotensin II, vasoactive intestinal polypeptide, KCl, BaCl₂, and MgCl₂. However Toyoshima et al. [5] suggested that the increase in intravesical pressure that occurs during stimulation with cholinergic agonists involves the participation of cholinergic M-2 receptors, and that the responsiveness to acetylcholine is reduced by aging. Other authors have also found a decrease in the force generated by aged strips of bladder smooth muscle: Munro and Wendt [6] found that the maximal force generated by the aged muscle strips decreased, and that the maximal velocity of shortening was significantly lower in strips from aged animals as compared with those from young adults. The effects of nerve stimulation appear to have been largely neglected, but Sneddon and McLees [7] found that contractile responses to nerve stimulation during the neonatal period became much less in adulthood in rabbits.

The size of the innervation received by the bladder has also been examined in a few studies: Gilpin et al. [8] found that the amount of nerve per mm² of muscle decreased with age, and occurred to the same extent in males and females. Warburton and Santer [9] studied the pattern and density of calcitonin gene-related peptide (CGRP)-containing afferent nerve terminals in the bladder, and found that this was unchanged in old age. In addition, these workers presented evidence which suggested a diminution in the sympathetic control of the urinary tract in aged rats. There appear to be no reports on the effects of aging on afferent function, and this could be important because it is known that the bladder afferents determine the onset of normal micturition.

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The present experiments were performed to investigate the pressure-volume relationships in young adult and aged rats, and the possibility that there are changes in the sensory or motor innervation of the viscus during aging.

**MATERIALS AND METHODS**

*General preparation.* Male Wistar rats weighing 255–320 g (aged 2–3 months) and 360–430 g (aged 26–29 months) were used in this study. The animals were obtained from the stock of laboratory animals of our Institute of Gerontology. Laboratory chow and water were provided ad libitum and rats were kept in a 12 h/12 h dark-light cycle, at a temperature of 22±2°C and a relative humidity of approximately 60% before experiments. The animals were anesthetized using solutions of chloralose and urethane. Two regimes were used: A, in which the initial loading doses of urethane and chloralose were 500 and 50 mg/kg, i.p., respectively; and B, in which the initial loading doses of urethane and chloralose were 90% of those given in regime A. In both regimes, maintenance doses of 50 mg/kg urethane and 5 mg/kg chloralose were given intravenously as required, as judged by changes in arterial pressure, heart rate, and respiration, and by the onset of a flexor withdrawal reflex to pinching. Jugular venous, carotid arterial, and tracheal cannulae were inserted, and the animal was artificially ventilated. Arterial pressure and heart rate were recorded continuously on a polygraph (RM-6000, Nihon Kohden, Tokyo). End-tidal CO₂ and O₂ levels were checked intermittently. Small volumes of 4% Ficoll 70 (Pharmacia Fine Chemicals AB, Uppsala) were given intravenously to keep systolic pressure above 70 mmHg when necessary. Body temperature was controlled at 37–37.5°C using a heating pad and an infrared lamp controlled by feedback from a temperature probe in the esophagus. Gallamine triethiodide (20 mg/kg, i.v.; Sigma, St. Louis, MO) was given before the start of nerve stimulation; additional doses were given as appropriate, after checking the depth of anesthesia.

*Cystometry and responses to pelvic nerve efferent stimulation.* Laparotomy was performed and the bladder was exposed and kept moist with warm saline. A catheter was inserted into the bladder via the anterior urethra, and intravesical pressure was recorded continuously on the polygraph. After emptying the bladder, cystometry was performed by infusion of isotonic saline at a speed of 170 μl/min, and the pressure was recorded continuously using a polygraph. The pressure and volume at which reflex micturition was initiated were noted. Infusion was stopped soon after reflex micturition was provoked. In experiments on 7 young adult and 4 aged rats, the pelvic nerve on one side was cut and prepared for stimulation of the cut peripheral end at 20 Hz of 10 s, using pulses of 10 V and 1.0 ms. This stimulus was given at a series of volumes, and the active and passive pressures were recorded at each volume. In young adult and aged animals, when larger volumes were reached, there was sometimes some basal tonic activity in the bladder. The pressure at the start of an induced contraction was noted, along with the baseline pressure.
after the end of the contraction; the mean of the pre- and post-contraction baseline pressures was used in the graphical presentations.

**Pelvic nerve afferent recording.** In experiments on 7 young adult and 8 aged animals, multiunit afferent activity was recorded from the cut peripheral end of the pelvic nerve proximal to the major pelvic ganglion and about 10 mm from the bladder, using bipolar platinum-iridium wire electrodes, and a preamplifier with a time constant of 0.01 s (S-0476, Nihon Kohden). Nerve activity was measured as mass discharges counted every one or 5 s with a spike counter (ATAC-3700, Nihon Kohden), and recorded on the polygraph.

**Sensitivity to distending pressures and volumes.** In 4 experiments on young adult and 5 experiments on aged rats, distension of the bladder to a series of known pressures was accomplished by changing the height of a saline-filled reservoir attached to the bladder cannula, so as to investigate the pressure sensitivity of the pelvic afferents. In experiments on 4 young adult and 4 aged rats, the effects of a slow infusion of isotonic saline were also studied, to test the volume sensitivity of the afferents. In each case, the spike discharge was recorded in every 1 or 5 s, and the pressure/volume conditions were noted.

Graphs showing the relationship between spike discharge and intravesical pressure were constructed for all animals, and the relationship between spike discharge and volume was calculated by two methods: (a) using the cystometric data for each animal, which had been obtained earlier in the experiment, and (b) by observing the spike rate during infusions into the bladder at a constant rate; the data presented refers to the latter method, but the results from the method in (a) were similar. From these graphs of spike rate versus pressure or volume, it was possible to estimate the threshold pressure and volume at which the afferent discharge increased. The slope of the response to pressure and volume was also compared by using the spike discharge rate at 600 mmH₂O as 100% in normalized graphs. Thus, the response to distending pressures and volumes in young adult and aged animals could be compared relative to this internal standard.

**Denervations.** In 3 young adult and 4 aged animals, denervation of pelvic and hypogastric nerves was performed bilaterally by cutting the hypogastric nerve as it entered the bladder near its base, and the pelvic nerve proximal to the major pelvic ganglion. This experiment was done to reveal the contribution of the innervation to the mechanical properties of the bladder.

**Statistics.** Data were expressed as mean ± SEM. Statistical significance was determined by the Mann-Whitney U-test.

**RESULTS**

1. **Body weight and bladder weight**

   Bladder weight was measured in 5 young adult and 4 aged animals, whose body weights were 298 ± 9 and 395 ± 13 g, respectively. The weight of the bladder increased with age, being 93 ± 9 mg at 2–3 months and 165 ± 18 mg at 26–29 months of age.
2. **Cystometry**

Cystometry was performed so as to observe the passive and active mechanical properties of the bladder.

**Cystometrograms from young adult animals.** In young adult animals, the slope of the pressure-volume curve began to increase at volumes of 0.3–0.7 ml, and reflex micturition appeared at this volume (or less in some instances). Figure 1A shows one cystometrogram generated in a young adult rat with bladder innervation intact, and the filled circles in Fig. 1C show the mean results of 4 young animals. The micturition threshold, the pressure at which micturition contractions commenced during the cystometrogram was \(183 \pm 35\) mmH\(_2\)O in 4 young adult rats. The bladder volume at the start of micturition was \(0.37 \pm 0.06\) ml in the young adult rats. Denervation did not produce any significant change in the pressure-volume curve, except that reflex micturition contractions were absent after denervation (Fig. 2A).

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**Fig. 1.** A, B: Cystometrograms from a young adult rat (A) and an aged rat (B) showing changes in intravesical pressure (mmH\(_2\)O) during the infusion of saline at 170 µl/min. In the young adult, a reflex micturition contraction occurs at a volume of about 0.3 ml. In the aged animals, the cystometrogram is characterized by the long plateau of low pressure during filling of the large capacity bladder. C: Averaged cystometric data of individual trials (1–3 trials/rat) from 4 young adult animals (filled circles: mean ± SEM), and from 4 aged animals (open circles).

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Cystometrograms from aged animals. In the aged animals, the pressure-volume curve plateaued at low volumes, and the pressure remained low at bladder volumes between 0.2 and 2.0 ml. At higher volumes the cystometergram became steeper. Micturition contractions were present at volumes over 1.5 ml in the animals anesthetized with anesthetic regime B. There was no essential difference between the cystometergrams using regimes A and B except for the occurrence of micturition contractions. Cystometric data from one aged animal is shown in Fig. 1B, and the mean results of 4 aged animals are shown by the open circles in Fig. 1C. The micturition threshold was $228 \pm 23$ mmH$_2$O in the aged animals. The volume at the start of micturition in the aged rats was $2.11 \pm 0.27$ ml. Denervation of the bladder produced little change in the pressure-volume relationship, apart from the absence of micturition contractions (Fig. 2B).

Comparison of cystometergrams in young adult and aged animals. In order to quantitate the shift to the right in the cystometergrams of aged animals, the volume of the bladder at 200 mmH$_2$O was measured; in the young adult animals, the mean volume was $0.33 \pm 0.05$ ml and in the aged animals it was $1.75 \pm 0.26$ ml. The significance of the shift to the right in the aged animals was $p < 0.005$.

3. Effects of pelvic nerve efferent stimulation—active and passive pressure generated at different bladder volumes

The effects of supramaximal stimulation of the efferent fibers of the pelvic nerve proximal to the major ganglion were observed in 7 young adult and 4 aged animals. The average of the pressures before and after the contraction was used as an estimate of passive pressure, i.e. that attributable to the viscoelastic properties of the bladder and each volume. The active pressure was defined as the peak pressure during the nerve stimulation. The same stimulus was used while the volume of the bladder was increased by infusion of isotonic saline in steps of 0.1 ml. Active and passive pressure-volume relationships were constructed in each case. Infusion was
stopped when the bladder produced reflex micturition. Figure 3 shows typical examples of the change in bladder pressure associated with bladder contraction during stimulation of one transected pelvic nerve. Figure 4 shows a comparison of the active (peak) and passive (basal) pressures achieved at different distending volumes in young adult and aged animals. The aged rats often were unable to elevate bladder pressure by the amounts seen in the young adult animals, at low or high bladder volumes. In these animals, one pelvic nerve was sectioned, but the difference between the passive pressure volume relationship seen in young adult and aged animals persisted. Following unilateral pelvic nerve section, however, the active pressures generated by nerve stimulation could be seen, and these showed that the aged animals rarely generated the levels of intravesical pressure seen in young adults. For example if the baseline pressure of 200 mmH₂O was chosen arbitrarily, the mean volume in the young adult animals was about 0.6 ml, and the active pressures approached 600 mmH₂O. In the aged animals, the passive pressure occurred at a volume of about 1.3 ml, and the peak pressure was 510 mmH₂O.

4. Pelvic nerve afferent activity

Pelvic nerve afferent sensitivity to distending pressures. Figure 5 shows the responses of mass afferent activity in pelvic nerve afferents in a young adult and an aged rat to distending the bladder using a reservoir to apply the desired pressure changes. Figure 6A shows the mean results at each pressure. The threshold of the
Fig. 4. The mean active and passive pressures in young adult and aged animals during stimulation of the cut peripheral end of a pelvic nerve. Nerve stimulation was applied as shown in Fig. 3; the active (peak) pressure (triangles) was measured at a series of volumes, and compared with the passive pressure (the mean of the baseline pressure before and after the contraction; circles) at each volume used. The graph shows the mean of individual trials (1-3 trials/rat) and SEM at each volume from 7 young adult rats (filled symbols) and 4 aged rats (open symbols).

Fig. 5. The illustration shows the relationship between mass afferent activity recorded in the peripheral cut end of one pelvic nerve at a series of different pressures. The bottom bar shows the bladder pressure generated by raising a reservoir of saline to the appropriate pressure. The afferent response is shown for a young adult animal (upper), and for an aged animal (lower).

most sensitive pelvic nerve afferents to distending pressures was between 100 and 200 mmH₂O in the young adult and the aged animals. In approximately half of the preparations in the young adult and aged groups, the relationship between dis-
charge frequency and pressure was linear. In the others, the discharge rate plateaued at pressures of about 200–600 mmH₂O. Thus, the mean results in young adult and aged animals shows a tendency to plateau and both results were almost identical. Further, an attempt was made to normalize the responses using the spike rate at 600 mmH₂O as 100%. When this was done (Fig. 6B), it was clear that there was no significant difference between the responses of the young adult and aged groups in the ability of these afferents to signal intravesical pressure; again the relationship was slightly curvilinear.

**Pelvic nerve afferent sensitivity to distending volumes.** In animals in which the pressure had been increased by raising the height of a reservoir attached to the bladder cannula, the relationship between impulse frequency of pelvic afferents and bladder volume was calculated from the relationship between impulse frequency and pressure, and the pressure-volume curve was obtained in the same animal. The slope of the volume-response relationship was much less steep in the aged rats, but the threshold volume of the most sensitive fibers was similar in the young adult and the aged rats.

In animals in which the volume of the bladder had been increased at a constant rate using a syringe pump, the relationship between the afferent spike rate and vesical volume was observed directly on the polygraph. The top records in Fig. 7A and B show two examples of the responses of pelvic nerve afferent preparations to distending volumes in young adult (in A) and aged (in B) animals. The lower records shows two examples of the cystometrograms in the young adult (in A) and aged (in B) rats. It is clear, however, that the pelvic nerve afferent preparations from the aged rat are not as good at signaling bladder volume as the young adult one.
Fig. 7. A: The rate of afferent discharge in the whole pelvic nerve during bladder distension in a young adult animal. The top trace shows the rate of discharge in pelvic nerve afferent fibers, and the bottom trace shows the cystometrogram which was recorded simultaneously. B: The same data, but from an aged animal.

Fig. 8. A: Volume sensitivity of pelvic nerve afferents. Data recorded from afferents recorded in the peripheral cut end of one pelvic nerve in 4 young adult and 4 aged rats. The graph shows the mean rate of individual trials (1–3 trials/rat) at each volume. B: The same data has been normalized: the spike rate recorded at 600 mmHg has been regarded as 100%. The mean rate of discharge at each volume is displayed.

Figure 8A shows meaned responses of the pelvic nerve afferent preparations at a series of bladder volumes in young adult and aged animals. In Fig. 8B, the results have been normalized using the same algorithm as mentioned previously (taking the
spike rate at 600 mmH$_2$O as 100%). It is clear that the volume-response relationship was shifted to the right in the aged animals. When individual responses were analyzed, the mean volume at which 50% of the increase in discharge occurred was $0.46 \pm 0.08$ ml in the young adult animals and $1.26 \pm 0.40$ ml in the aged animals; the significance of the difference was $p < 0.01$.

DISCUSSION

The conclusions drawn from these experiments differ from those of Chun et al. [2, 3]. These differences are probably due to the degree of hypertrophy of the aged bladder in the two experiments. These authors used the bladders of Fischer 344 rats aged 5–7 months and 22–24 months in in vivo and in vitro measurements, whereas the current experiments were performed in vivo on Wistar rats aged 2–3 and 26–29 months. In both experiments, the bladder weight increased with age, by about 80% in the current experiments, and by about 20% in those of Chun et al. [2]. This difference of the degree of hypertrophy of the aged bladder appears to be due to the difference in strain of rats. A difference arises, however, in the pressure and volume at which micturition contractions occurred in the two groups of animals. In the current experiments, micturition contractions occurred at similar pressures in young adult and old animals, but the volume threshold for micturition was higher in the aged group of rats. This contrasts with the results of Chun et al. [2]; there was a systematic difference between their experiments and ours in that the pressure at micturition in their experiments was lower than that found in ours. This applies to both groups of animals, viz. young and aged, and may be related to the rate of filling of the bladder [10]. In their experiments, the volume of the bladder at micturition was actually lower in the aged animals (0.45 ml) than in their young adults (0.58 ml), whereas in the present experiments it was 0.37 ml in the young adult and 2.11 ml in the aged animals.

So there is a profound and essential difference between the data presented by Chun et al. [2, 3] and the present work: we see grossly enlarged bladders, they did not. The most obvious difference between young and old cystometrograms in the present experiments is the steady plateau in the pressure-volume curve which persists up to volumes of around 2 ml in the old animals. Chun et al. [3] pointed out that the average plateau pressure significantly decreased with age, a finding with which we are in agreement (Fig. 1). The long plateau phase and the shift to the right in the cystometrogram during aging are unaltered by denervation: this indicates that the sympathetic system, which is known to relax the bladder and shift the cystometrogram to the right [11] is not specifically involved in maintaining the high bladder volumes seen in aged animals. The profound shift in the pressure-volume curve seen in aging must depend on viscoelastic changes in the bladder tissue, rather than on receptive relaxation attributable to its innervation. This conclusion is opposite to that reached by Chun et al. [2], who suggested that the primary changes were in the innervation. The difference in opinion probably relates
to the differences between the groups of rats in the two studies mentioned at the start of the discussion. We do see changes in the innervation with age, but these coexist with profound changes in the mechanical properties of the bladder itself. In this paper, the changes in innervation relate to (a) the decreased sensitivity of the afferents to changes in volume, and (b) to changes in the contractile response that may be related to changes in the size of the efferent innervation. Gilpin et al. [8] found that the number of neurons in the bladder wall diminished with age; and there is a corresponding increase in the muscarinic receptor density. The former halved during aging; the latter increased [12]. So the changes in the contractile responses reported in this paper, in particular the reduction in active pressure may be related to these changes in efferent innervation.

The results of experiments on the motor responses of young and old rats are inconclusive in that there are suggestions that the sensitivity to acetylcholine may be decreased in elderly animals. Toyoshima et al. [5] obtained evidence of a change in M-2 receptor function, but Saito et al. [4] found no age-related changes in the response to acetylcholine. There appear to be no reports of changes in the response to nerve stimulation, and the present experiments were performed to investigate whether any differences between the motor responses of young and old rats could be defined. Because the contractile response of vesical muscle depends on the initial length of the muscle, active and passive pressures were measured at a series of volumes. It is clear that the responses of young and old animals differed considerably. According to the Laplace Law, the pressure generated should be proportional to radius and to wall tension; despite the increase in radius with age, the pressure generated diminishes, suggesting a considerable reduction in contractile ability. The reasons for this may lie with the smooth muscle: it is not known how smooth muscle cell length, or activation change during aging. Another possible reason might be a change in the innervation: aging is known to be associated with neuronal death, and in the peripheral nervous system, there is good evidence that motor axon numbers decrease with age, and that this is associated with terminal sprouting [13]. The most striking effect, apart from the long plateau in the cystometrogram, is the reduction in contractile capacity in many of the bladders from elderly rats. The difference between passive and active pressure decreased at the highest bladder volumes, particularly in the aging rats, and this may be one reason why bladder emptying may be incomplete.

The mass activity recorded from distension-sensitive afferents in the pelvic nerves of young and old rats begins at similar levels of pressure and the slope of the stimulus response function in the two groups is not significantly different. However, the range of volumes which is detected by these afferents is very different in the two groups: insofar as the slope of the volume-response function can be monitored using mass recordings, it appears much less steep in the aged animals, and the afferent response near the micturition threshold appears similar in young and old animals. It is apparent that the afferent neurons are behaving similarly in young and old animals as far as pressure reception is concerned, but these sensory

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endings behave very differently as volume receptors. Volume sensitivity is reduced significantly in old age, whereas pressure sensitivity is not. It is possible that the shift to the right in the volume sensitivity of pelvic nerve afferents in the elderly rats may explain the increase in residual volume that has been reported.

The mechanism underlying the complete emptying of the bladder during micturition is unknown. The electrophysiological correlate of the micturition reflex, i.e. the evoked activity in pelvic nerve efferents following electrical stimulation of pelvic nerve afferents, is modulated by bladder pressure. However, activity in this reflex pathway increases dramatically when intravesical pressure or volume is rising; but diminishes profoundly if not completely within seconds of a fall in bladder pressure or volume. The nature of the mechanisms that maintain the voiding phase of bladder activity until the bladder is empty are largely unknown. But if they fail, an increase in the residual volume of urine can result, and it is known that its occurs in elderly humans [14, 15]. It is possible that a change in afferent activity might be involved in the failure to completely expel urine from the bladder, e.g. if volume sensation is compromised.

These results differ from most other experimental studies on aged rats in that our rats were older and the changes in the lower urinary tract were more marked. The changes in bladder function during aging can be attributed to (a) changes in the mechanical properties of the bladder, (b) changes in the contractile properties of its smooth muscle during efferent nerve stimulation, and (c) changes in the ability of its afferent innervation to sense bladder volume. These changes may explain the increase in residual volume, the inability to postpone voiding and the decrease in flow rate seen in elderly humans.

We would like to thank Ms. A. Suzuki, Ms. H. Kashiwagi, and Ms. A. Kimura for their expert help and support during the experimental work described in this paper. J.F.B.M. would like to thank Tokyo Metropolitan Institute of Gerontology for a visiting Professorship. This work was partially supported by Grant-in-Aid for Encouragement of Young Scientists (No. 07770053 to H.H. and No. 07770054 to S.U.) from the Ministry of Education, Science and Culture of Japan.

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