Impaired Bladder Function in Aging Male Rats

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Abbreviations and Acronyms

BC = bladder capacity

B_{com} = bladder compliance

BP = baseline pressure

CRC = carbachol response curve

EC_{50} = drug concentration needed to produce 50% of calculated maximum

EFS = electrical field stimulation

E_{max} = calculated maximum effect

IMP = interMP

MF = micturition frequency

MP = micturition pressure

MV = micturition volume

pEC_{50} = negative logarithm of EC_{50}

RV = post-void residual volume

SA = spontaneous activity

TP = threshold pressure

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Purpose: The prevalence of bladder dysfunctions increases with age. In humans it is difficult to separate changes related to exogenous factors from those directly related to the aging process. Some confounding variables can be avoided by studying age related changes in an animal model. We evaluated the impact of age on bladder function in vivo and in vitro, and characterized the corresponding morphological changes.

Materials and Methods: Young (4 to 6 months old) and old (older than 28 to 30 months) male Fischer/Brown Norway rats were used in the study. Cystometric studies were done in conscious, freely moving rats. After cystometry tissue strips from the bladder body were used in in vitro studies of muscarinic receptor activation and electrical field stimulation, and histological examination.

Results: Old rats had higher bladder weight than young rats but the bladder-to-body weight ratio did not change. We noted significant age related differences in 8 of 10 cystometric parameters. Old rats had increased bladder capacity, post-void residual volume, micturition volume and frequency, baseline and intermicturition pressure, and spontaneous activity but decreased micturition pressure. Bladder strip responses to carbachol and electrical field stimulation were significantly lower in old than in young rats. Histological examination revealed urothelial thinning, lower muscle mass and higher collagen content in the bladders of old vs young rats.

Conclusions: Physiological aging alters bladder function in male rats even when external factors remain constant. Thus, in old rats bladder capacity, post-void residual urine and spontaneous activity are higher, and responses to muscarinic receptor stimulation and electrical field stimulation are lower than in young rats. Such changes correspond to findings in aging human bladders, supporting the view that the Fischer/Brown Norway rat is a useful model in which to study age related bladder function changes.

Key Words: urinary bladder, physiology, aging, urodynamics, male

RECENT reviews in the clinical literature document a clear age related decrease in the ability of the bladder to fill, store and empty.[1–3] However, the relationship of aging to other external influences on the detrusor, such as nervous system disease, vascular supply or lower urinary tract smooth muscle, remains poorly understood.[4–6] More insight into the impact of these pathophysiological conditions on bladder function is needed but equally important is the development of relevant preclinical models in which these external influences can be reduced.

Several groups have evaluated age related changes in rodent bladder function but only a few used in vivo cystom-

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etry to characterize the changes.[7,8] In vitro studies in old vs young rat bladder tissue have yielded contradictory results that may be attributable at least partly to strain specific differences. For example, in Fischer 344 rats muscarinic receptor mediated detrusor contraction was increased,[9] unchanged[10,11] or decreased.[12] In Wistar rats muscarinic receptor mediated bladder contraction was unaltered.[8,13] Studies of Sprague-Dawley® rats showed decreased muscarinic receptor mediated detrusor contraction.[14–16]

Thus, despite the number of studies of age related changes in bladder function few reports have assessed in vivo and in vitro parameters in the same rat. To this end we evaluated the impact of aging on bladder function in vivo and in vitro in male Fischer/Brown Norway rats (Harlan™). The Fischer/Brown Norway rat strain is often used in aging studies[17,18] since these rats have an increased life span compared to that of other rodent strains, and a decrease in age related pituitary, testicular and kidney pathology compared to Sprague-Dawley rats of similar ages. We report our initial observations on age related changes in urodynamic parameters, detrusor contractile responses to muscarinic receptor and EFS, and bladder wall histological characteristics in young and old Fischer/Brown Norway rats.

MATERIALS AND METHODS

Animals

In this study we used 15 young (4 to 6 months old) and 15 old (28 to 30 months old) male Fischer/Brown Norway rats from the National Institute on Aging colony, National Institutes of Health. All experimental protocols were approved by the Wake Forest University Health Sciences animal care and use committee. Animals were maintained on a 12-hour light/12-hour dark cycle in polycarbonate cages with free access to Purina™ rat chow and water. After surgery rats were kept 1 per cage to prevent damage to implanted catheters. Animal well-being was supervised daily during the entire experimental period. Animals with large tumors or decreased body weight indicating underlying disease were not used in these studies.

Urodynamics

Bladder catheter implantation. Rats were anesthetized by intraperitoneal injection of 50 mg/kg pentobarbital. The abdomen was opened through a lower midline incision and a PE-50 polyethylene catheter (Clay-Adams, Parsippany, New Jersey) was implanted into the bladder through the dome. The indwelling catheter was tunneled subcutaneously and exited through an orifice in the back of the neck.

Cystometric recording. Cystometric recording was done without anesthesia 3 days after bladder catheterization, as previously described.[19] The bladder catheter was connected to a pressure transducer, an ETH 400 transducer amplifier (CB Sciences, Dover, New Hampshire) and a PowerLab®/8e data acquisition board. Bladder pressure and MV real-time display and recording were done on a computer using PowerLab software, version 5.1. The conscious rat was placed without restraint in a metabolic cage, which also enabled urine volume measurement by a fluid collector connected to a force displacement transducer. Room temperature saline was infused into the bladder at 10 ml per hour.

Recorded or calculated cystometric parameters were 1) BC—RV at the previous micturition plus the volume of infused saline at micturition, 2) MV—expelled urine volume, 3) RV—BC − MV, 4) BP—lowest average pressure recorded between voids, generally recorded shortly after voiding, 5) TP—pressure at which the micturition contraction is initiated, 6) MP—maximum bladder pressure during voiding, 7) IMP—mean pressure between 2 micturitions, 8) SA—IMP − BP, 9) B_{com}—(BC/(TP − BP) and 10) MF—number of voids per hour (see table).

Organ Bath Studies

Tissue preparation. After cystometry the rats were sacrificed and the bladders were carefully removed. Full-thickness strips of the lateral part of the bladder wall were saved for histology. After removing the urothelium/suburothelium the bladder body was cut longitudinally into 4, 4×10 mm strips, which were placed in 15 ml tissue bath chambers. The chamber was filled with Krebs solution maintained at 37C and bubbled continuously with a mixture of 95% O_2 and 5% CO_2, resulting in pH 7.4. The strips were suspended between 2 L-shaped hooks by silk ligatures. One hook was connected to a movable unit, allowing adjustment of passive tension, and the other was connected to an FT03C force transducer (Grass Instruments, Rockland, Massachusetts). Isometric tension was recorded using the same digital PowerLab system as for cystometric measurement. After mounting the strips were stretched to 2 gm passive tension and allowed to equilibrate for 60 minutes before further experiments.

Electrical field stimulation. EFS was done using 2 platinum electrodes placed on each side of the strips and controlled by an S88 stimulator (Grass Instruments) delivering single square wave pulses at select frequencies. Train duration was 5 seconds, pulse duration was 0.8 milliseconds and the stimulation interval was 2 minutes. Electrode polarity was shifted after each pulse by a polarity changing unit. The strips were continuously stimulated from low to high selected frequencies (1 to 32 Hz).

Carbachol response curve. Cumulative steady-state CRCs were constructed on isolated bladder strip preparations by adding carbachol at ½ log increments at concentrations of 3×10^{-9} to 10^{-4} M.

Histology

Bladder wall histological data were analyzed in 4 μm sections stained with hematoxylin and eosin or Masson's trichrome stain. With the latter method in formalin fixed, paraffin embedded sections collagen fibers stain blue, nuclei stain black and cellular material (muscle and cytoplasm) stain red. Urothelial thickness and collagen content (blue areas) were analyzed using Image-Pro® AMS