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Old age does not affect shortening velocity or content of contractile and cytoskeletal proteins in the rat detrusor smooth muscle

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Abstract The influence of old age on mechanical properties of the urinary bladder was investigated using smooth muscle strips from urinary bladders of control (14–16 weeks) and old-age (104 weeks) female Sprague-Dawley rats. Bladder weight of the aged rats had increased by about 30%. The maximal shortening velocity and stiffness in skinned activated urinary bladder fibers from old animals were unchanged compared to controls. The relative content of intermediate filament proteins to actin and the relative content of myosin to actin was unchanged. The concentration of myosin was unchanged (about 6.5 μg/mg wet weight). The results suggest that old age is not associated with pronounced changes in the cellular contractile and cytoskeletal proteins or in the mechanical properties of the contractile machinery. The age-related changes in mechanical properties previously reported for intact smooth muscle from urinary bladder are most likely due to alterations in the activation systems.

Key words Bladder smooth muscle · Intermediate filaments · Age · Actin · Myosin · Force-velocity relation

Introduction

The aging process affects the function of the lower urinary tract in several ways. Common clinical problems include voiding dysfunction secondary to benign prostate hyperplasia and urinary incontinence. Other reported dysfunctions are incomplete voiding with residual urine, increased voiding frequency, lowered flow-rates and reduced compliance [13]. The bladder emptying is dependent on the activation of the bladder smooth muscle cells and the contraction of the cells being sufficiently forceful and sustained. This means that the response is dependent on function at all levels of excitation-contraction coupling in the smooth muscle: from receptor activation, second messenger systems, calcium release or influx and finally the activation of the contractile machinery.

It has been reported that the aging process results in degeneration of the smooth muscle cells and nerve axons, and that these changes correlate with voiding dysfunction and reduced contractility in the aged human bladder [6]. Several functional changes have been reported to occur in the aging detrusor smooth muscle, e.g., decreased responses to cholinergic [15] and β-adrenergic agonists [16]. The sensitivity to acetylcholine does not change with the age of the detrusor [17, 19], although an increase in the response has been reported for the bladder base [9, 17].

Very little is known about changes in the mechanical properties in the aged urinary bladder smooth muscle. Studies on intact smooth muscle strips from the bladder of old rats have shown decreases in maximal shortening velocity (V_{max}), isometric force and metabolic tension cost [15]. It is not possible in intact smooth muscle to evaluate at what step(s) in the process of activation and contraction the age-dependent changes have appeared, which leads to the decreases in V_{max} and tension cost.

The objective of the present investigation was to compare urinary bladder smooth muscle from adult and aged rats with regard to the content of contractile and cytoskeletal proteins and to investigate force-velocity relations and series-elastic stiffness in the maximally activated state. These experiments focus on the contractile machinery and were made using maximally activated, chemically skinned muscle strips, thus eliminating factors, e.g., variations in activation, which could influence the force-velocity relationship in intact cells.

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Material and methods

Animals and preparations

Female Sprague-Dawley rats were held in standard animal housing facilities, maintaining controlled environment and fed a normal diet. Urinary bladders were obtained from adult (14–16 weeks) and old (104 weeks) animals. The rats were killed by cervical dislocation and the bladders were quickly taken out and placed in ice-cold physiological salt solution, gently blotted between two filter papers and weighed. Smooth muscle strips from the mid-section of the bladder were carefully dissected and the mucosa was removed with scissors. The muscle strips were skinned in a Triton X-100 solution [3] and stored at –15°C in a glycerol-containing solution for later experiments. The mechanical experiments were performed on the skinned preparations according to the protocol described by Arner and Hellstrand [3]. Intact smooth muscle strips were also frozen in liquid nitrogen and stored at –80°C and later used for biochemical determinations.

Quick release experiments

Quick release experiments [2] were used to determine V_{max} for the skinned preparations of both old and adult animals. Aluminium foil was wrapped around both ends of the preparations. The muscle strips were mounted horizontally in an organ bath (22°C) between an isotonic lever and a force transducer (AE801, Senso-Nor, Horten, Norway). The muscle was stretched to a length where passive tension was just noticeable. The afterload of the preparation was adjusted by varying the load on the lever, which could be clamped or released with electromagnetic relays. The experiments were performed on preparations maximally activated by thiophosphorylation of the regulatory light-chains [1]. The muscles were incubated for 15 min in an ATP-free rigor solution containing Ca^{2+} (pCa 4.5), 0.5 μM calmodulin and 2 mM ATP-γ-S and thereafter contracted by introducing MgATP at pCa 9.0. Releases (15–20) to different afterloads were made at the plateau of the contraction. After each series of releases the preparations were again thiophosphorylated for 10 min. Two series of releases were done on each muscle preparation. Force and length were digitized at 1 kHz using a computer equipped with an Analog Devices (Norwood, MA, USA) RTI-800F A/D board. The shortening velocity decreases with time after the release [3], and therefore the velocity was determined at a standardized point in time (100 ms) after the release. Afterload (P) and velocity (v) data were fitted to the Hill equation [7] in the form: $v = b(1-P/Po)/(P/Po + a/Po)$, where Po is isometric force and a and b are constants. V_{max} was calculated as bPo/a. The stiffness, i.e., series elastic component, was determined as described by Arner [2]. The relation between the relative length change (dL/L) and the relative force (dP/P) immediately after the release was fitted to the logarithmic function $dL/L = 1/Kln(P/Po-B)/A)$ where A, B and K are constants. The constant K (describing the relation between stiffness dP/dL and force) was used to quantitate stiffness.

Determination of contractile and intermediate filament proteins

Sodium dodecyl sulfate (SDS)-gel electrophoresis was performed essentially as described by Laemmli [10]. Frozen bladder tissue was crushed and homogenized in SDS buffer [50 mg tissue wet weight/ml; composition: 25 mM TRIS (hydroxymethyl) aminomethane HCl (pH 6.8), 2% SDS, 5% mercaptoethanol and 10% glycerol]. The homogenate was boiled for 2 min and centrifuged. The supernatant was then removed and the gels (8% SDS polyacrylamide) were loaded with different amounts of muscle extract and standard protein (chicken gizzard myosin). After electrophoresis the gels were stained overnight with 1% (wt/vol) Coomassie blue in 40% methanol and 10% acetic acid and destained with 39% methanol and 10% acetic acid until the background was clear. The gels were scanned (GS-30 densitometer, Hoefer, San Francisco, CA, USA) and the area under the peaks of actin, myosin and intermediate filament proteins was evaluated and used for quantification of the proteins.

Statistics

Values are given as means ± SEM with the number of animals within parenthesis. Comparisons were made using Student's t-test for unpaired data with $P < 0.05$ as the significance level.

Results

The weight of the control rats was 274 ± 18 g ($n = 6$) and that of the old rats was 305 ± 8 g ($n = 7$). Bladder weight of the old rats increased significantly (122 ± 5 mg, $n = 7$) compared with the controls (91 ± 5 mg, $n = 6$) ($P < 0.001$). The relation bladder weight to weight of the rat also showed a significant difference (control: 0.033 ± 0.001%, $n = 6$; old: 0.040 ± 0.002%, $n = 7$ with $P < 0.01$).

Force-velocity relation and stiffness

Table 1 shows force-velocity relationships from control and old-age rat bladder preparations. Two determinations were performed on each preparation and the average value was used as a representative value for the muscle. No difference in V_{max} (in muscle lengths per second) could be detected between the control group (0.21 ± 0.02 ML/s, $n = 6$) and the old-age group (0.21 ± 0.02 ML/s, $n = 7$). The components b and Po/a of the Hill equation [7] describing the shape of the curve were 0.046 ± 0.004 and 0.214 ± 0.027 in the control group and 0.053 ± 0.005 and 0.292 ± 0.029 in the old preparations. The stiffness constant K, describing the dependence of stiffness on active force, was 48 ± 2 and 52 ± 5 in the control and the old preparations, respectively.

Table 1 Properties of urinary bladders from control (adult 14- to 16-week-old) and old-age (104 weeks) rats. Shortening velocity (in muscle lengths per second) and the stiffness parameter K were determined in quick release experiments. Intermediate filament proteins (IFP, mainly desmin), myosin/actin ratio and myosin concentration were determined using 7% SDS-polyacrylamide gel electrophoresis

	Control	Old age
Bladder weight (mg)	91 ± 5 (6)	122 ± 4 (9)
Shortening velocity (ML/s)	0.21 ± 0.02 (6)	0.21 ± 0.02 (7)
Stiffness	48 ± 2 (6)	52 ± 5 (7)
IFP/actin	0.14 ± 0.02 (6)	0.14 ± 0.07 (7)
Myosin/actin	0.46 ± 0.03 (6)	0.54 ± 0.06 (7)
Myosin (μg/mg wet weight)	6.6 ± 0.06 (5)	6.3 ± 0.05 (4)

Determination of contractile and intermediate filament proteins

The ratio of the content of intermediate filament proteins to actin remained unchanged (controls: 0.14 ± 0.02, n = 6; old-age: 0.14 ± 0.02, n = 7). The ratio of myosin to actin increased slightly but insignificantly (control: 0.46 ± 0.03, n = 6; old-age: 0.54 ± 0.06, n = 7). Quantitative analysis of the myosin content showed, in control bladder, 6.6 ± 0.6 μg/mg tissue wet weight (n = 5), and in old bladder, 6.3 ± 0.5 μg/mg wet weight (n = 4).

Discussion

In whole bladder cystometrograms in vitro, old age has been reported to be associated with an increased compliance [5]. The structural or functional changes in the bladder wall, responsible for this alteration, are unknown. In both the aged female human [21] and rat [6] urinary bladder, an increased amount of collagen has been found which seems to be contrary to what is expected if the amount of collagen determines the bladder elasticity. In comparison, during growth of the rat urinary bladder induced by a partial urinary outflow obstruction, a decreased amount of collagen and an increased wall stiffness is found [14]. Increased cellular amounts of the intermediate filament proteins, mainly desmin, have been correlated with growth in vivo, both in the hypertrophying portal vein [11] and in the hypertrophying urinary bladder [12]. The role of these cytoskeletal proteins is unknown at present. It is possible that they in part contribute to the elasticity of the smooth muscle tissue. Although we did not perform an extensive characterization of the tissue elasticity in the aged bladder, our finding that the series elasticity parameter was unchanged is in general agreement with the unaltered relative content of the intermediate filament proteins.

A decreased bladder contractility is common in elderly people [6] even in the absence of detrusor instability or outlet obstruction. In the aged human urinary bladder with clinically impaired contractility, a degeneration of the smooth muscle with several structural changes including a loss of alignment of myofilaments has been observed [6]. The decreased contractility could in part be due to a decreased amount of contractile proteins in the bladder wall secondary to this smooth muscle cell degeneration.

There seems to be a correlation between maximal active force and maximal shortening velocity (V_{max}) in an individual smooth muscle strip [22]. This relation is not necessarily true if strips from different bladders are compared. Differences in content of contractile proteins might affect force but not V_{max}. These parameters reflect the number of active cross-bridges and the cross-bridge turnover rate, respectively. The decreased contractility in the elderly, in the sense of being less able to produce a powerful and sustained contraction, is thus not necessarily correlated to the maximal shortening velocity of the detrusor muscle cells. However, the study by Saito et al. [18] suggests a decreased rate of contraction in old age. The rats included in our study represent very late stages of life and the slight increase in bladder weight might reflect a contractile dysfunction, although we did not perform any cystometrical experiments or measurements of active force production to directly demonstrate changes in bladder contractility. On the other hand, the unchanged relative content of myosin/actin and the unchanged amount of myosin suggest that the aged rat urinary bladder does not reduce the content of myofilaments. The lower force and decreased number of myofilaments seen in other studies [6, 18] might therefore not be correlated with a general reduction in the concentration of contractile proteins in old age.

In intact bladder strips from aging rats, Munro and Wendt [15] have shown a decreased V_{max}, indicating a slower cross-bridge cycling under unloaded conditions. However, the metabolic tension cost, considered to reflect cross-bridge turnover under isometric conditions, was unaltered [15]. Similar results have been reported for hypertrophying detrusor smooth muscle of rats with a 10-day urinary outflow obstruction [4, 20]. In this model the active force per smooth muscle cross-section area and the amount of myosin decreased [12]. Other changes in hypertrophic rat urinary bladder strips include a reduced shortening velocity, correlated with changes in the light chain and myosin heavy chain isoforms [20]. The bladder weight increased slightly with old age. This might be related to a small hypertrophy in response to increased micturition volumes and rates together with a decreased passive compliance [5]. In contrast with results from obstructed bladders, we find that the V_{max} measured in maximally activated muscles is identical in control and old-age preparations. Even though a small hypertrophy is observed in old age, the decreased V_{max} and altered myosin content associated with the hypertrophic bladder smooth muscle does not seem to occur in the aged rat urinary bladder. This difference between the hypertrophic smooth muscle of aged animals and animals with urinary outflow obstruction could be due to the difference in the time-course of the hypertrophy process or in the extent of hypertrophy.

In smooth muscle the level of activation has been reported to influence the maximal shortening velocity [3]. In the skinned maximally thiophosphorylated muscle fibers, used in the present investigation, the level of activation of the contractile machinery is held maximal. The unchanged V_{max} in the old-age preparation thus suggests that the previously reported decreased V_{max} in field-stimulated intact fibers from old rats [15] is due to alterations in the activation systems or calcium homeostasis. In aged rabbit bladder a decreased number of Ca^{2+}-channel antagonist binding sites have been found [23]. Also, in the myocardium of the aged hamster a decreased density of voltage-sensitive Ca^{2+} channels has been reported [8]. These studies suggest that an age-induced change of the Ca^{2+} sensitivity of the urinary

bladder smooth muscle might be responsible for the previously reported decreased maximal shortening velocity [15] and force responses [18] in intact preparations. Contrary to these changes in activation pathways, the amounts of contractile and structural proteins and their mechanical behavior remain essentially unaltered with old age.

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