Functional and Molecular Dysregulation of Lower Urinary Tract Smooth Muscle Resulting in Underactive Bladder in Old Mice

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Context

- Bladder underactivity is a highly prevalent condition in both men and women, particularly in the elderly, which undoubtedly impairs patient's quality of life [1].
- The International Continence Society has defined underactive bladder (UAB) as prolonged urination time with or without a sensation of incomplete bladder emptying, usually with hesitancy, reduced sensation on filling, and slow stream [1]. Current understanding of the pathophysiology of UAB is limited and efficient pharmacological treatments are lacking.
- Recently, age-related changes in bladder of old female mice (27-30-month old) were identified through functional and molecular studies [2].

Objectives

We investigated here the functional and molecular alterations of the contractile and relaxant machinery in the lower urinary tract smooth muscle of 18-month female mice, focusing on muscarinic and adrenergic receptors in bladder as well as the nitric oxide (NO)-soluble guanylyl cyclase (sGC) pathway in urethra.

Methods

- Female young (3-month old) and old (18-month old) C57BL/6 mice were used.
- Cystometry was performed in urethane-anesthetized mice [3]. Briefly, bladders were filled at a constant rate (0.6 mL/h) and intravesical pressure was recorded for 45 min.
- Neurogenic contractions were evaluated by electrical-field stimulation (EFS) in isolated bladders (1-32 Hz).
- Concentration-response curves to contractile (carbachol) and relaxing agents (mirabegron) in isolated bladders, as well as the contractile responses in urethral smooth muscle (phenylephrine) were also employed.
- mRNA expressions of muscarinic receptors (M2 and M3 subtypes), adrenergic (α-1A-, β2- and β3-adrenoceptors), sGCβ1, and neuronal nitric oxide synthase (nNOS) were determined by RT-PCR, and results normalized to actin mRNA expression levels.
- Statistical analysis: Comparisons among the groups were evaluated using Student's *t* test.

Results

In the cystometric study (Fig.1), young mice showed regular micturition cycles whereas old mice showed an atypical voiding pattern characterized by an incapacity to produce regular bladder contractions and emptying during a 45-min observation.

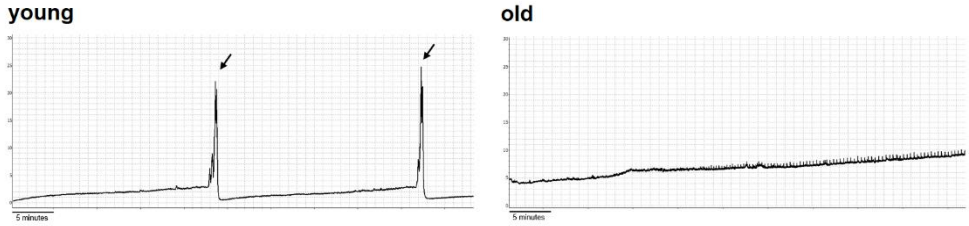

Figure 1. Representative cystometric traces of young and old female mice.

In isolated bladders, EFS produced frequency-dependent bladder contractions in young and old groups, but the responses were significantly lower in old compared with the young group (Fig. 2a; P < 0.05). The bladder contractions to carbachol were also reduced in old compared with young group (Fig. 2b). Bladder responses to the selective β3-adrenergic agonist mirabegron were enhanced in old compared with young mice (Fig. 2c).). In isolated urethra, phenylephrine produced higher contractions in old compared with young group (pEC50: 6.46 ± 0.05 and 5.87 ± 0.21, respectively; P<0.05).

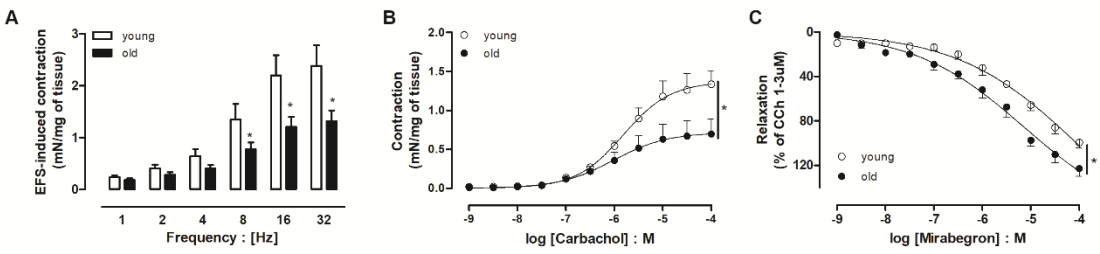

Figure 2. Contractile responses induced by electrical field stimulation (EFS; A) and muscarinic agonist carbachol (B), and relaxation induced by the β3-adrenergic selective agonist mirabegron (C) in isolated bladder from young and old mice. Data represents means ± SEM, n = 6 animal/group. *P<0.05 in comparison to young.

We next evaluated the muscarinic and adrenergic mRNA expressions in bladder and urethra (RT-PCR). In bladders, the mRNA assays revealed no differences for the muscarinic M2 and M3 receptors between young and old groups. The β2 adrenergic receptor mRNA also remained unchanged, but a significantly higher expression of β3 adrenergic receptors in old mice was found (P<0.05; Fig. 3a). Urethra of old mice also displayed a significant increase in α-1A adrenergic receptor mRNA expression (P<0.05; Fig. 3b). Because activation of nNOS-sGC-cGMP signaling pathway is crucial to promote urethral relaxations during the micturition cycle, we evaluated both nNOS and sGC mRNA expressions. We found a marked decrease in sGCβ1 and nNOS in urethra of old compared with young mice (P<0.05; Fig. 3c,d).

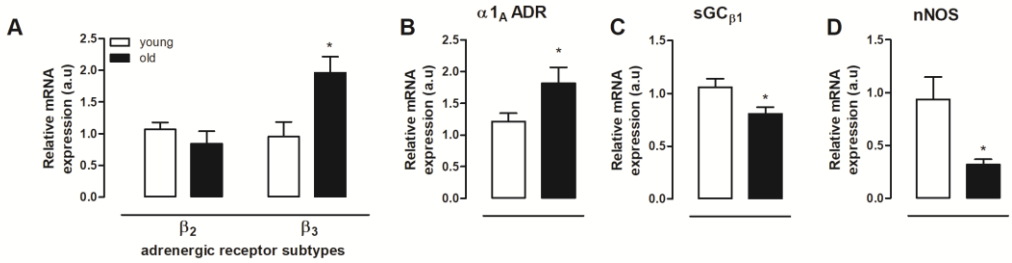

Figure 3. mRNA expression for β2 and β3-adrenergic receptors (A) in isolated bladder of young and old mice. mRNA expression for α1a-adrenergic receptor (α1a-ADR; B), soluble guanylyl cyclase (sGC; C) and neuronal nitric oxide synthase (nNOS; D) in isolated urethra from young and old mice. Data represents means ± SEM, n = 6 animal/group. *P<0.05 in comparison to young.

Concluding message

Our data show that underactive bladder in old mice (cystometry) is accompanied by reduced bladder contractions to EFS and carbachol. Our findings that muscarinic M2 and M3 are unaltered in bladders of old mice indicate that signaling downstream muscarinic receptors may be implicated in the impaired contractions. On the other hand, mirabegron-induced bladder relaxations are increased in bladders of old mice which is accompanied by higher mRNA expression of β3-adrenoceptors, indicating an enhanced relaxing tone in these animals that might contribute to UAB. When looking to the urethral smooth muscle in old mice we found increases of phenylephrine-induced contractions and α1-adrenergic mRNA expression, accompanied by reduced relaxing mechanisms (nNOS and sGCβ1).

Taken together, our results demonstrate the presence of an age-associated UAB caused by an atonic and over relaxed detrusor smooth muscle and an overactive urethra, resulting in impairment of emptying efficacy.

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