

## AGED RELATED CHANGES IN PURINERGIC SIGNALLING, AFFERENT FIRING AND RECEPTOR EXPRESSION IN THE MOUSE BLADDER.

### Hypothesis / aims of study

Overactive bladder syndrome (OABS) is a debilitating condition which poses serious adverse effects on quality of life. OAB is characterised by the symptoms of urgency, frequency and nocturia and in some patients is associated with urinary incontinence. Although OAB and urinary incontinence are not exclusively diseases of aging, both have increased prevalence in the aging population, although the mechanisms underlying this are poorly understood. Our hypothesis is that altered sensory signalling may be a contributing factor. The urinary bladder urothelium was classically considered to be an inert barrier; however it is now clear that the urothelium is an important regulator of bladder sensory function. The aim of this study was to investigate the effect of aging on sensory nerve function and gene expression in the mouse bladder.

### Study design, materials and methods

Control mice (aged 3 months) and aged mice (12 and 24 months) were used in this study. Mice were sacrificed humanely in accordance with UK legislation. The afferent nerve response to bladder filling was measured using an in vitro model previously described (1). The bladder and surrounding tissue was placed in a recording chamber circulated with oxygenated (95% O<sub>2</sub>, 5% CO<sub>2</sub>) Krebs-bicarbonate solution at 35°C. The urethra and dome were catheterised. Multiunit branches of the pelvic and hypogastric nerve were drawn into an electrode and afferent nerve activity was recorded. Bladders were distended to a maximal intravesical pressure of 50 mmHg with isotonic saline. To determine ATP release from the urothelium, samples of the infusate were collected and ATP release was determined using the luciferin-luciferase ATP assay. Bladder compliance was gauged by the pressure-volume relationship. In gene expression studies, bladders were excised and the urothelium/suburothelium was dissected from the underlying muscle. RNA was extracted from both muscle and urothelium/suburothelium tissues and cDNA was synthesized. Quantitative gene expression analysis was performed using TaqMan-based Real Time PCR assay for purinergic receptors belonging to P<sub>2</sub>X family (P<sub>2</sub>X<sub>1</sub>-P<sub>2</sub>X<sub>7</sub>).

### Results

Distension of the bladder evoked a pressure-dependent increase in afferent firing which remained consistent following repeated distensions with saline. The stimulus-response function of bladder afferents was significantly altered with age. Bladders from 24 month old mice (n=4) exhibited a significantly higher afferent responses to distension compared to bladders from 3 month old mice (P<0.01 2-Way ANOVA and Bonferroni post-test). Moreover compliance was significantly increased in bladders from both 12 month and 24 month old mice compared to controls (P<0.0001 2-Way ANOVA and Bonferroni post-test). ATP detected in intraluminal samples was significantly higher in bladders from 24 month old mice compared to controls (4.3 ± 0.7 nM, n=9 vs 16.69 ± 6.34 nM, n=5, P<0.01 Student's t test) suggesting that ATP release from the urothelium/suburothelium increases in age.

In urothelium/suburothelium from 12 month and 24 month old animals, q-PCR revealed a significant down-regulation of P<sub>2</sub>X<sub>2</sub> (fold change=5 and 5), P<sub>2</sub>X<sub>3</sub> (fold change=2 and 4), P<sub>2</sub>X<sub>5</sub> (fold change=10 and 9) and P<sub>2</sub>X<sub>6</sub> (fold change=13 and 16) receptors (n=5 and 4) compared to controls (P<0.01 ANOVA with Bonferroni, n=5). However surprisingly in detrusor muscle P<sub>2</sub>X<sub>1</sub> receptor expression was moderately increased (fold change =2) in bladders from 24 (n=3) month old mice compared to control (n=6).

### Interpretation of results

The increase in mechanosensation observed in this study suggests that in aging, bladder afferent nerves may exhibit hypersensitivity. This phenomenon could also be secondary to alterations in bladder compliance and detrusor tone which was observed at both 12 and 24 months or could result due to increased bioavailability of ATP from the urothelium/suburothelium (evident at 24 months). Increased ATP may act at downstream targets on the sensory nerve terminal to enhance afferent transmission. Together these functional changes could induce alterations in purinergic receptor expression in the detrusor and urothelium/suburothelium as a compensatory mechanism.

### Concluding message

Taken together these data suggest that ageing results in an increase in bioavailability of ATP which may lead to aberrant urothelial-afferent function, increased mechanosensitivity and alterations in purinergic receptor gene expression. Further studies are required to elucidate the mechanisms involved.

### References

1. Daly D, Rong W, Chess-Williams R et al. Bladder afferent sensitivity in wild-type and TRPV1 knockout mice. *J Physiol* 2007; 583:663-74.

### Disclosures

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