

## BLADDER PAIN INDUCED IN RATS BY CHRONIC STRESS IS MEDIATED BY PROLONGED STIMULATION OF ALPHA 1A ADRENOCEPTORS.

### Hypothesis / aims of study

Chronic water avoidance stress (WAS) induces sustained bladder hyperalgesia and urinary frequency in rats, lasting over a month after exposure to stress[1,2]. WAS rats also show a decreased response threshold to mechanical stimulation of hind paw suggestive of referred hyperalgesia [1,2]. Thus this model seems to be valuable for studying BPS/IC in rats. Interestingly, chronic stimulation of alpha 1A adrenoceptors (AR) expressed in bladder primary afferents and urothelial cells increased both pain response to suprapubic mechanical stimulation and the number of voiding contractions. This study aims to investigate whether WAS induces bladder pain and increases voiding frequency by chronic stimulation of bladder alpha1A AR.

### Study design, materials and methods

WAS was induced in adult female Wistar rats by placing the animals on a pedestal in the centre of a cage filled with water at room temperature for 1h/day, for 10 consecutive days.

Lower abdomen pain was measured by the mechanical pain threshold after stimulation with von Frey filaments at baseline and after WAS. The same procedure was performed in WAS animals treated with Silodosin (WAS+Silodosin), an alpha 1A AR antagonist, daily during the WAS period. Controls, WAS and WAS+Silodosin rats were anaesthetised and their urine was collected and pooled. Then, bladder frequency was determined by cystometry. The bladder of all animals were harvested, sectioned, stained with Hematoxylin-Eosin to evaluate the percentage of disrupted urothelium (lack of superficial urothelial cells) or stained with toluidine blue to investigate the presence of mast cells in the mucosa.

T-test and Kruskal-Wallis followed from Dunn's Multiple Comparisons Test were used for group comparisons.

### Results

Noradrenaline/ml urine in controls was 0.22 nmol while in WAS rats was 8.79 nmol ( $p < 0.001$ ). Urinary noradrenaline levels in WAS+Silodosin rats was 4.33 nmol.

Baseline mechanical pain threshold was  $40 \pm 19$  g. In WAS rats threshold decreased to  $11 \pm 9$  g ( $p < 0.05$ ). Another group of animals presented a basal pain threshold of  $45 \pm 26$  g. When subjected to WAS+silodosin, pain threshold did not change ( $43 \pm 29$  g,  $p > 0.05$ ).

Controls had  $0.43 \pm 0.11$  voiding contractions/minute. WAS increased this number to  $2.12 \pm 0.19$  ( $p < 0.05$ ). WAS rats treated with silodosin had bladder voiding contractions similar to controls ( $0.60 \pm 0.42$ ,  $p > 0.05$ ).

The urinary bladder from control animals did not present urothelial damage. However, the bladder from WAS animals presented a  $6 \pm 4$  % of damaged urothelium. Silodosin administration prevented the urothelial disruption.

The bladder's mucosa of control animals had  $15 \pm 4$  mast cells/mm<sup>2</sup>. WAS animals had  $32 \pm 3$  mast cells/mm<sup>2</sup> on their bladder's mucosa ( $p < 0.05$ ). The bladder of WAS+silodosin animals had  $16 \pm 5$  mast cells/mm<sup>2</sup> in the mucosa.

### Interpretation of results

WAS increased the urinary levels of noradrenaline, indicating that a chronic adrenergic overactivity occurs in WAS. As silodosin treatment prevented the development of bladder pain, bladder histological changes and the increase of the number of voiding contractions, alpha 1A AR should mediate the consequences of chronic adrenergic stimulation in WAS.

### Concluding message

Bladder pain, urothelial disruption, bladder mastocytosis and hyperactivity induced by WAS is due to an increased release of noradrenaline leading to an abnormal stimulation of alpha 1A AR. These findings may be relevant for the treatment of interstitial cystitis.

### References

1. Urology. 2011 Oct;78(4):967.e1-7.
2. Physiol Behav. 2015 Feb;139:541-8.

### Disclosures

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