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NEURAL MECHANISMS OF BLADDER OVERACTIVITY IN EARLY STAGES OF BLADDER OUTFLOW OBSTRUCTION

Hypothesis / aims of study

Bladder outflow obstruction, secondary to an enlargement of the prostate gland due to benign prostatic hyperplasia, results in bladder overactivity. It has been suggested, that in early stages of obstruction in mice, bladder overactivity is mainly due to afferent dysfunction (1). We have recently characterized major classes of sensory neurons in the bladder based on their mechano- and chemosensitivity (2, 3). The aim of this study was to determine whether bladder overactivity in early stages of obstruction was due to enhanced excitability of bladder afferents and/or enhanced function of efferent nerves and the detrusor muscle.

Study design, materials and methods

Partial bladder outflow obstruction was produced by placing a ligature around the urethra in C57BL/6 female mice for 7-12 days. Short-term (2 hrs) voluntary urination, in freely moving conscious mice, was analyzed by counting void stains on filter paper. In anesthetized mice, rhythmic reflex bladder contractions were evoked by isovolumetric distension (0.15-0.2ml) of the bladder. Single unit stretch-sensitive bladder afferents were recorded by conventional extracellular recording technique *in vitro*. The spike frequency adaptation method was used to determine the electrical excitability of afferents. The ability of the afferent fibre to follow the electrical stimuli (1-80 Hz, 0.5ms, 20 stimuli) plotted for each frequency, giving an excitability index for particular afferent. Intravesical pressure responses to repetitive electrical field stimulation (EFS, 1-30Hz for 3 s, 0.15 ms) applied via two Pt plates were determined in isolated whole bladder *in vitro*.

Results

The frequency of conscious voiding was significantly increased after bladder outflow obstruction (2.8 ± 0.2 per 2hrs, n=5), compared with control (1.6 ± 0.3 per 2 hrs, n=10, $P<0.05$), while the voiding volume was not significantly changed (177 ± 15 ul, n=5, versus 246 ± 32 ul, n=10, NS). In anesthetized mice, distension of the bladder with a phosphate buffer solution activated rhythmic reflex bladder contractions, which were blocked by TTX (0.3 uM). The frequency of reflex bladder contractions was significantly increased after obstruction (1.14 ± 0.14 per min, n=4), compared with control (0.64 ± 0.1 per min, n=5, $P<0.05$), while their amplitude was not significantly changed (12.6 ± 1.44 cmH_2O, n=4, obstructed and 15.2 ± 3.48 cmH_2O, n=5, control, NS). In the presence of 3 uM of nicardipine, the excitability indexes of low threshold stretch-sensitive afferents in response to repetitive (1-80Hz) stimulation were significantly higher in obstructed preparations, compared with control (2 way ANOVA, $P<0.05$). At 20 Hz, 77 ± 11% (n=5) of spikes followed stimulation in obstructed preparations, while in control only 47± 6% (n=4, $P<0.05$). In obstructed mice, intravesical pressure responses to EFS (normalized against 80 mM KCl-induced contraction) were significantly reduced: response to 30Hz of EFS was reduced by 59 ± 11% ($P<0.01$). The cholinergic component (assessed after application of 3 uM hyoscine) was reduced from 45 ± 18% (n=4, control) to 26 ± 2.8% (n=5, obstructed, $P<0.001$), while the purinergic component of hyoscine-resistant contraction (assessed after consecutive application of 30 uM PPADS) was not changed (30 ± 4.9%, n=4, control) and 28 ± 4.4% (n=5, obstructed). At these stages of obstruction, responses to 80 mM KCl did not differ between control and obstructed bladders, suggesting no significant changes in muscle function. This was supported by the hematoxylin and eosin staining technique which revealed no significant muscle hypertrophy in early stages of obstruction (n=4).

Interpretation of results

The data indicate that in early stages of obstruction, there are clear signs of bladder overactivity, such as increased frequency of voiding and reflex bladder contractions. Low threshold stretch-sensitive afferents show increased excitability, while function of the detrusor muscle, or efferent nerves, were not elevated at this time.

Concluding message

The results of the present study suggest that sensitization of low threshold stretch-sensitive afferent endings is a likely cause of bladder overactivity in early stages of bladder obstruction. Future studies are needed to determine which factors evoke the sensitization of stretch-sensitive bladder afferents after bladder outflow obstruction.

References

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