

DIFFERENT TYPES OF MECHANORECEPTORS IN THE BLADDER

Hypothesis / aims of study

Sensory neurons represent an attractive target for pharmacological treatment of bladder overactivity, which affects ~17% of the population in the USA and Europe (1,2). However it is still unclear how many classes of sensory neurons are involved in signalling bladder function and their mechanism of activation. The aim of this study was to distinguish the different classes of mechano-sensitive sensory neurons innervating the guinea-pig bladder *in vitro* and to investigate whether stretch-sensitive mechanoreceptors transduce mechanical stimuli directly (via mechano-sensitive ion channels) or via release of chemicals (in particular ATP) from urothelial cells.

Study design, materials and methods

Adult male guinea pigs (total N=27), weighing between 240-280 g, were humanly killed. The urinary bladder was removed and opened into a flat sheet. In most cases full thickness, small (14 mm x 15-20 mm) flat sheet preparations were studied with the mucosa uppermost. "Close-to-target" extracellular recordings were made from axons in fine nerve trunks entering the bladder trigone via platinum electrodes. Single units were discriminated by amplitude and duration using Spike Histogram software (AD Instruments). Preparations were stretched longitudinally by a microprocessor-controlled stepper motor at 1000 $\mu\text{m/s}$ for distances of 1-4 mm and held for 10 s, at 3-4 minutes intervals.

Results

Several distinct classes of bladder mechanoreceptors could be distinguished by responses to stretch, von Frey hair compression, stroking of receptive fields, applications of chemical stimuli to the mucosa) during electrophysiological recordings from guinea pig bladder afferents *in vitro*. These include a two stretch-sensitive afferents. First, most abundant, were "tension-mucosal mechanoreceptors" (n=29, N=23), which could be activated by stretch, mucosal stroking with von Frey hair (0.1-2 mN) and by hypertonic solutions (1M mannitol and 500 mM NaCl) applied locally to their receptive fields in the mucosa. Second, were "muscle mechanoreceptors" (n=9, N=9), which responded to stretch behaving as "in-series tension receptors", but which lacked responses to mucosal stroking (0.1-2 mN) or hypertonic stimuli. Third, we have also recorded stretch-insensitive afferents – "mucosal mechanoreceptors" (n=12, N=10) most of which could be transiently activated by mucosal stroking (0.1-2 mN), by compression (0.5-2 mN) and by hypertonic solutions (2 of 6 units tested, N=6) applied to their receptive field in the mucosa.

In mucosa-free preparations, the non-selective P2X/P2Y purinoreceptor antagonist, PPADS (30 μM) did not affect stretch-induced firing by low threshold muscle mechanoreceptors (n=4, N=4) but significantly inhibited α,β -methylene ATP (30 μM)-induced contractions (to $8\pm 3\%$ of control, N=4, $P<0.05$) and associated afferent firing (to $25\pm 9\%$ of control, n=5, N=4, $P<0.05$). Transduction by low threshold stretch-sensitive mechanoreceptors does not appear to involve exocytotic transmitter release since it occurs in Ca^{2+} -free (with 1mM EDTA and 6 mM Mg^{2+}) Krebs solution (n=6, N=4).

The majority of tension-mucosal mechanoreceptors were briefly (5-11s) activated by α,β -methylene ATP (1mM for 1min, 6 out of 7 units) applied locally to their receptor fields in the mucosa. PPADS (30 μM) and suramin (100 μM) did not affect stroking (2 mN)-induced firing of tension-mucosal mechanoreceptors ($95\pm 3\%$, n=4, N=4 and $110\pm 7\%$, n=4, N=3, respectively). Capsaicin (3 μM , n=5, N=5) applied to mucosa did not activate these afferents. Only 2 out of 9 tension-mucosal units (N=9) were slightly activated by hypotonic stimuli (10-25 mM NaCl), in contrast to hypertonic stimuli (500 mM NaCl) by which 13 of 14 (N=13) units were activated. Mucosal mechanoreceptors were not sensitive to capsaicin (3 μM , n=4, N=4) or to α,β -methylene ATP (1mM for 1min, n=3, N=4). Suramin (100 μM) did not affect stroking (2 mN)-induced firing of mucosal mechanoreceptors ($94\pm 4\%$ of control, n=3, N=3).

Interpretation of results

We have distinguished several distinct populations of sensory neurons based on their responses to stretch, von Frey hair, stroking/compression of receptive fields, applications of chemical stimuli to the mucosa. They are likely to represent different classes of bladder afferents with different mechanisms of activation. In the present study on the guinea-pig bladder, the non-selective P2X/P2Y antagonists, PPADS and suramin, did not affect mechanotransduction by low threshold mechanoreceptors. The discrepancies between these results and previous findings obtained on mouse bladder (3) are probably due to the particular classes of pelvic afferents studied, species and experimental condition differences.

Concluding message

Using a novel *in vitro* bladder preparation, at least three different classes of bladder mechanoreceptors have been distinguished: (i) stretch-sensitive afferents - tension-mucosal mechanoreceptors and (ii) muscle mechanoreceptors, (iii) stretch-insensitive mucosal mechanoreceptors. The present study has also suggest that mechanotransduction by low threshold mechanoreceptors in the guinea-pig bladder does not require exocytotic transmitter release or endogenous ATP, suggesting direct mechanism of mechanotransduction by this type of afferent. Endogenous ATP may modulate activity of muscle and tension-mucosal mechanoreceptors. The use of *in vitro* extracellular recordings with anterograde labelling and subsequent immunohistochemical methods will make possible detailed structural-functional characterization of major classes of bladder afferent neurons.

References

- (1) BJU Intl 87, 760-766, 2001.
- (2) Word J Urol, 20, 327-336, 2003.

(3) J Physiol, 541, 591-600, 2002.

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ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by Animal Welfare Committee of Flinders University of the South Australia