52

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MODULATION OF SUB-UROTHELIAL MYOFIBROBLAST RESPONSES TO SENSORY MODULATORS

Hypothesis / aims of study

The cellular physiology of bladder urge and urgency is poorly understood. One mechanism is that afferents are activated either by substances released from the urothelium when it is stretched, such as ATP, or by changes to the local environment, such as an acidosis. Support of this comes from the fact that mice lacking purinergic ($P2X_3$) or $TRPV_1$ receptors show micturition reflexes of reduced gain (1,2). In addition, a functional syncitium of suburothelial myofibroblasts has been described which make intimate connections with the urothelium and afferent nerves. These cells are excited by exogenous ATP and propagate signals throughout the suburothelium, and it has been hypothesised they are an intermediate stage in the sensory process (3). To test further this hypothesis the effects of interventions that may modulate bladder sensations on myofibroblast responses have been recorded.

Study design, materials and methods

Myofibroblasts were isolated from guinea-pig bladder mucosa by collagenase disruption. Cells were loaded with Fura-2 to measure the intracellular [Ca^{2+}], $[Ca^{2+}]_i$ and held under voltage-clamp (-60 mV) to record membrane currents. Cells were superfused (pH 7.4, 37°C) with a CO_2/HCO_3^--buffered solution to record responses to ATP, or a HEPES/MES-buffered solution when changes to pH were made. Data are mean±S.D, significance between data sets was examined by Student's *t*-test; at $p<0.05$. All chemicals are from Sigma, except Glivec which was a gift from Novartis.

Results

Isolated cells responded to ATP (10-100 µM) by generation of an intracelluar Ca^{2+}-transient and an inward current. ATP responses were attenuated by the NO-donor Na nitroprusside (1 mM, 40±21% control, *n*=4). Pre-treatment with capsaicin (5-30 µM) also reduced the inward current (37±12% control, *n*=8) but by contrast had only a minor effect on the Ca^{2+}-transient (87±7% control). Reduction of pH (to between 4.5-5.5) generated similar responses to exogenous ATP. When two cells were pushed together, but without the formation of gap junctions (i.e. cell capacitance was unaltered) the magnitude of the responses to ATP and pH were enhanced almost two-fold. Moreover, the threshold ATP concentration or magnitude of pH change required to generate a response was also reduced. The augmentation of responses with cell pairs was abolished by 30-100 µM glivec (*n*=12).

Interpretation of results

Myofibroblasts respond to interventions that are postulated to be involved in normal sensory or nociceptive reponses. Moreover such responses can be attenuated by agents that eventually reduce sensory responses in the intact bladder, or neuromodulatory agents such as NO. Augmentation of the magnitude of these basic responses by cell pair formation, and the reduction of threshold at which ATP and low pH are effective, suggest that an intact network of cells is a powerful modulator of the action of these sensory signals. Glivec binds to tyrosine-kinase associated receptors that modulate intracellular signalling pathways, sometimes in association proteinas associated with adherens junctions. The ability of the agent to block responses augmented by cel pair formation suggests a novel pathway to modulate the effects of exogenous ATP and acidosis in the suburothelial sensory region of the bladder wall.

Concluding message

Suburothelial myofibroblasts respond to ATP and acidosis, agents associated with ultimate activation of sensory afferents. The ability to suppress myofibroblast responses by exogenous agents proposes novel pathways to modulate sensory responses arising from the bladder wall.

References

1 Nature. 2000; 407: 1011-1015.
2 Nature Neuroscience. 2002; 5: 856-860.
3 Journal of Physiology. 2004; 559: 231-243

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