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MODULATION OF UROTHELIAL FUNCTION AND AFFERENT SENSITIVITY BY NEUROKININ A.

Hypothesis / aims of study

Tachykinins are implicated in the mechanism that controls normal bladder function but the actions of these molecules within the bladder, and their specific sites of action have yet to be elucidated. Tachykinins are primarily contained within capsaicin sensitive primary afferent neurones (CSPAN's), and in the bladder these are found innervating the detrusor smooth muscle and, more densely, the sub urothelium (1). It has been shown that in the sub-urothelium of patients with idiopathic detrusor overactivity there is a significant increase in tachykinin immunoreactive fibres (1). Administration of neurokinin A (NKA) and neurokinin 2 (NK2) agonists into the bladder lumen have been shown to reduce bladder capacity, and increase micturition frequency in vivo (2), whilst NKA has also been shown to be released from capsaicin sensitive bladder nerves within the urothelium in response to muscle contraction and urothelial stroking (3).It is hypothesised that the urothelium is able to respond to neurokinins released from nerve endings innervating the urothelium, and through activation of the urothelial signalling pathway, the micturition reflex can be modulated in times of inflammation to cause overactive bladder and incontinence.The aim of this study is to show that urothelial cells themselves are able to respond to tachyknins and as a result of this, significantly alter detrusor muscle activity and afferent nerve output, which initiate the micturition reflex.

Study design, materials and methods

Mouse bladder afferent nerve activity was recorded using an in vitro preparation which enables the simultaneous recordings of afferent nerve firing and intravesical pressure. Saline or NKA (300nM) was continuously infused into the bladder lumen at 30µl/min whilst bladder distensions to an intravesical pressure of 30mm/Hg were performed every 10 minutes. Luminal contents were collected before (baseline) and after (stimulated) distentions and assayed for ATP and Acetylcholine (Ach) using commercially available kits. Primary mouse urothelial cells (PMUC's) were isolated from 12wk old mice, plated onto collagen coated coverslips and incubated overnight in serum-free media. Cells were loaded with Fura-2 AM (2µM) and excited alternately with 350 and 380nm wavelengths of light to measure intracellular calcium levels. Duplicate applications of NKA (300nM) 40 minutes apart were applied to cells with infusion of either NK1 antagonist CP9994 (1µM), NK2 antagonist GR159897 (300nM), or NK3 antagonist SB222200 (1µM) or DMSO control. All data are mean percentage±SEM of first response compared to ionomycin and compared using Student's t-tests as appropriate.RNA was harvested from 1 day cultured PMUC's, converted to cDNA and qPCR was performed using SYBR green fluorescence to identify NK1,2 and 3 receptors. Gel electrophoresis was also performed to ensure amplification products were of designed length.

Results

Intravesical NKA (300nM) caused a significant increase in the frequency (contractions/distension) 17.4±3.1 vs 6.8±1.7 (n=6, $p \leq 0.01$), and amplitude 1.8±0.4mm/Hg vs 0.7±0.2mm/Hg (n=6, $p \leq 0.05$), of individual spontaneous contractions. These increases in muscle activity are accompanied by a significant increase in afferent nerve activity at a physiological volume of 150µl (109±21.6imp/s vs 89±18.3imp/s (n=6, $p \leq 0.05$)) Fig 1a. These effects are not attenuated by the NK2 antagonist GR159897 (300nM). ATP and Ach were consistently released from the urothelium during distension. Following intravesical NKA, ATP release from the urothelium during distension was unchanged 6.2±1.5nM (saline) vs 5.9±1.1nM (300nM NKA). Ach release from the urothelium however, was significantly attenuated following NKA treatment at 435±85nM vs.180±19nM ($p \leq 0.01$) Fig 1b. Calcium imaging of PMUC shows urothelial cells were able to respond to NKA (300nM) with an increase in intracellular calcium at 11.6±0.9% (N=3, n=60) of the maximum ionomycin response. The initial calcium response to NKA was reduced by 85.6% following a second dose. Incubation with the NK2 receptor antagonist GR159897 (300nM) had no effect on second doses of NKA (300nM) Fig1d. In contrast, incubation with the NK1 and NK3 receptor antagonists reduced the response to 24.3 and 51% of the first dose respectively, suggesting these two receptors are functionally relevant in mediating urothelial responses to NKA. qPCR of PMUC reveals expression of all three tachyknin receptors, NK1, NK2, and NK3. With NK3 receptor expression approximately twice that of NK1 and 2 Fig 1c.

Interpretation of results

These data provide evidence for the role of neurokinins, released either from the suburothelial nerves or urothelium itself, in mediating the micturition reflex. Afferent firing and muscle contraction are the key components to maintaining continence and the present findings demonstrate that neurokinin A acting on the urothelium can alter both. These data have shown that neurokinins are able to activate the urothelium directly to increase intracellular calcium concentrations, but also significantly reduce the release of Ach, an important neurotransmitter within bladder physiology. It could be proposed that the increases in spontaneous muscle contraction and afferent output are a result of this reduced Ach, and thus provide a mechanism by which neurokinins acting on the urothelium can indirectly affect the micturition reflex. This data provides evidence towards the importance of the urothelium and its role in mediating continence via modulation of release factors. Interpretation of these results could also propose a mechanism by which neuronal inflammation of CSPAN's can directly influence urothelial function, and in turn, the maintenance of continence.

Concluding message

In the search for new, less invasive treatments for pharmacological treatments of incontinence, the ability to influence the primary outputs influencing micturition is essential. Neurokinin actions on urothelial cells provide such a mechanism via a novel route which has only now been fully investigated.

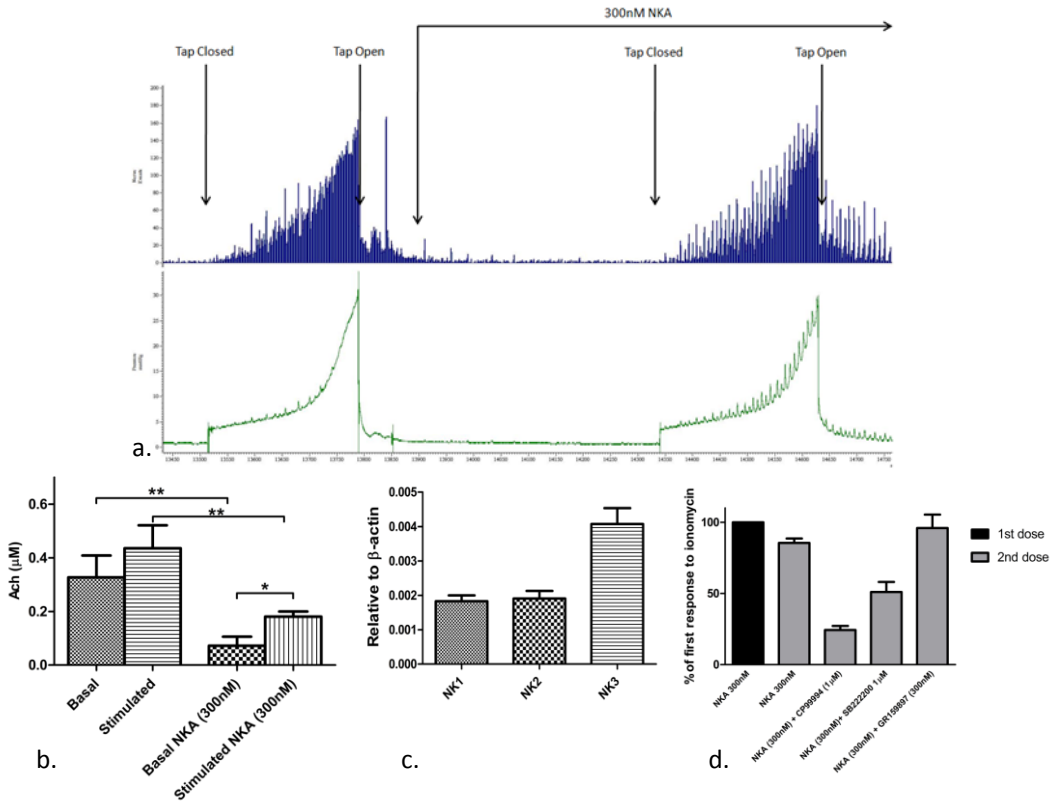

Fig 1. a, Increased phasic activity and associated afferent firing in the presence of NKA. b, changes in Ach release following intravesical NKA. c, relative expression of neurokinin receptors. d, relative effect of antagonists on NKA induced urothelial calcium.

References

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Disclosures

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