

## ROLE OF TRPV3 IN URINARY BLADDER FUNCTION AND SENSATION

### Hypothesis / aims of study

TRP channels have been shown to act as mechanosensory and pain receptors in a wide variety of organ systems. Despite its presence in the bladder, to date their pharmacologic modulation has not been shown to be effective in the treatment of overactive bladder and lower urinary tract symptoms. This study utilizes wild-type (WT) and TRPV3-knock out (KO) mice, coupled with novel drug compounds to clarify TRPV3's role in the regulation of bladder function and sensory signalling.

### Study design, materials and methods

Immunohistochemistry was used in a mouse model to localize TRPV3 in dorsal root ganglia (DRG) and the wall of the urinary bladder following treatment with an established outflow obstruction model. Isometric force contractile responses of bladder wall strips were measured following administration of TRPV3 specific agonist and antagonists, and additional studies were performed using TRPA1 antagonists as well as neurokinin (NK) receptor antagonists. Both WT and KO animals underwent testing with various TRP-family agonists and antagonists. Multifiber afferent nerve activity and cystometry were recorded in awake mice during bladder filling and micturition. The intravesical pressure and nerve activity were correlated during simultaneous bladder filling with 0.9% NaCl, then 0.25% acetic acid (AA) in normal saline, followed by TRPV3 specific drugs. Twelve-hour micturition frequency studies of KO and WT animals were performed using metabolic cages following an acclimation period 24-hours prior.

### Results

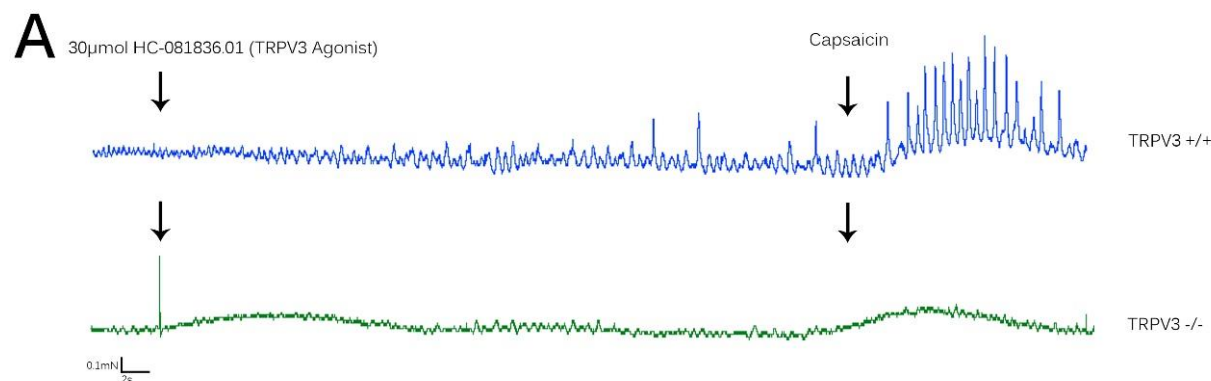
TRPV3 expression was documented in the urothelium, suburothelium, bladder smooth muscle, and L6 DRG neurons and there was increased expression-in animals after partial bladder outflow obstruction. TRPV3 agonists increased the amplitude of baseline phasic contractions in strips of bladder muscle wall (n=7, p < 0.01). This was potentiated with simultaneous TRPV3 and TRPV1 activation using capsaicin. TRPV3 suppression seen during the application of a TRPV3 antagonist had no effect on baseline contractility, however combination of TRPV3 and TRPA1 antagonists decreased detrusor muscle tone (n=5, p < 0.01). NK receptor antagonists suppressed phasic contractions during application of TRPV3 agonist (n=6, p < 0.05). Using awake cystometry, the increased intravesical pressure and voiding frequency seen with 0.25% AA infusion reversed towards normal with addition of TRPV3 antagonists (n=5, p < 0.01). Treatment with agonist and capsaicin on muscle strips from global TRPV3-KO mice demonstrated minimal change from baseline as compared to TRPV3-WT mice. (Figures A and B, n=4, p < 0.01). Adding TRPA1 antagonists to TRPV3-KO mice or TRPV3 and A1 antagonists to WT mice both suppressed the amplitude of phasic contractions (Figures C and D, n=4, p < 0.01). Micturition frequency studies demonstrated increased voiding frequency in TRPV3-KO mice (n=5, p < 0.01).

### Interpretation of results

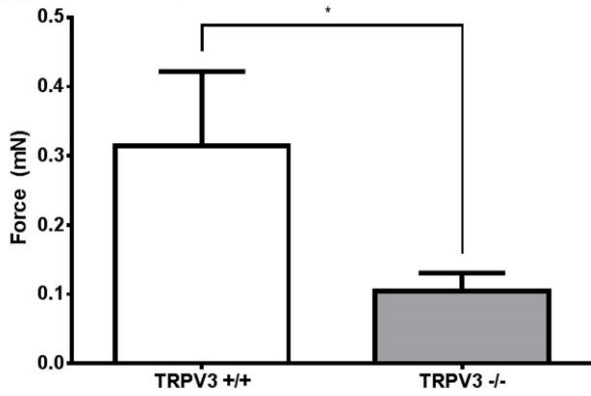
These data suggest that TRPV3 receptors play an integral role in bladder function and sensation. As demonstrated by immunostaining, mouse bladder and neurons following partial bladder outlet obstruction demonstrated increased TRPV3 expression, suggesting an underlying pathophysiology involving TRPV3. Further *ex vivo* functional studies involving myography demonstrated increased detrusor muscle contractility in response to TRPV3-stimulative drug compounds. Furthermore, activation of TRPV3 in addition to other channels potentiated bladder contractility. Downstream blockade of NK receptors involved in local pain pathways diminished this response, as did similar experiments involving TRPV3-KO mice. Awake *in vivo* cystometry recordings demonstrated similar bladder contractile activity in response to TRPV3 agonists. Based on this data, we believe that TRPV3 is involved in bladder function. Either a lack of TRPV3 or increased TRPV3 expression, affects the bladder's ability to contract efficiently, leading to an observed increase in urinary frequency.

### Concluding message

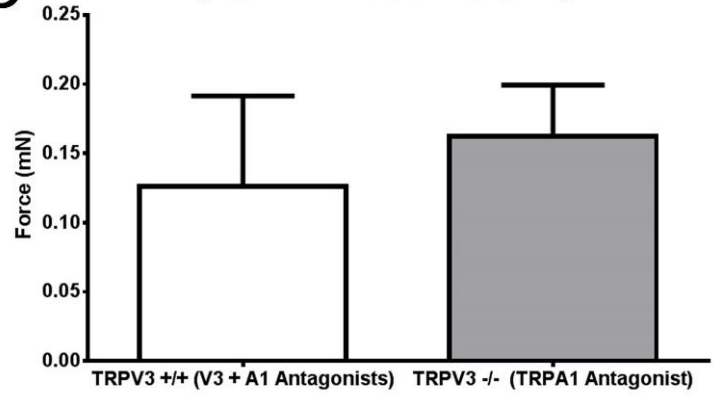
Presented data suggest that TRPV3 is present and has a functional role in the urinary bladder. Similar to the effects of TRPV1-specific capsaicin, TRPV3-specific compounds may exert their effects through modulation of neurokinin release, such as substance P. Moreover, the fact that TRPV3 exerts a suppressive effect on bladder phasic activity in combination with TRPA1, but not alone, suggests that multi-target therapy may prove effective for the treatment of OAB.



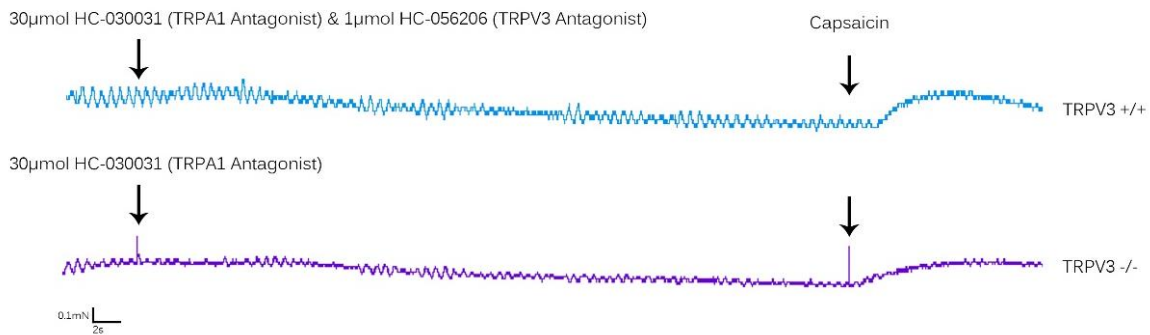
**B** Graph Summary of Phasic Contraction Amplitude During Application of Capsaicin (Figure A)



**C** Graph Summary of Phasic Contraction Amplitude During Application of Capsaicin (Figure D)



**D**



Disclosures

**Funding:** Department of Surgery University of Vermont **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Mouse **Ethics Committee:** University of Vermont Institutional Animal Care and Use Committee