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Grundy L[1], Daly D[2], Mansfield K[3], Chess-Williams R[1], Grundy D[2]

1. *Bond University, Faculty of Health Science and Medicine,* ***2.*** *Sheffield University, Department of Biomedical Science,* ***3.*** *University of Woolongong, Graduate School of Medicine*

SENSORY NERVE RESPONSES TO ATP ARE REGULATED BY TRPV1 RECEPTORS.

Hypothesis / aims of study

ATP is released from the urothelium during bladder filling and its actions at purinergic receptors on bladder sensory nerves is an essential function of normal voiding behaviour, disruption of which leads to bladder hyporeflexia and disrupted micturition [1]. The TRPV1 receptor, also present on primary afferent fibres, is consistently implicated in inflammation and pain [2] and a more recent role in signalling during bladder filling has emerged [3]. The aim of this study was to determine the nature of interactions between TRPV1 and P2X receptors in modulating afferent nerve activity.

Study design, materials and methods

Mouse bladder sensory nerve activity was recorded using an in vitro preparation which enables the simultaneous recordings of afferent nerve firing and intravesical pressure. αβMe-ATP (30μM) was administered to partially distended (12mmHg) bladders and afferent nerve activity and contraction responses were compared in the absence and presence of the TRPV1 antagonist capsazepine (10μM). Similar experiments were also performed in TRPV1 knockout mice. In another set of experiments the responses of the nerves was investigated by measuring intracellular calcium changes. Pelvic and hypogastric dorsal root ganglion neurones were isolated from TRPV1 knockout animals and age matched wild type controls. Cells were loaded with Fura-2 AM (2μM) and excited alternately with 340 and 380nm wavelengths of light to measure intracellular calcium levels. Responses to ATP and capsaicin were examined. All data are mean±SEM and were compared using Student's t-tests as appropriate.

Results

The purinergic agonist αβMe-ATP evoked a profound increase in afferent discharge (Figure 1) and a concurrent contraction which returned to baseline over 2-3min. In the TRPV1 knockout mice the afferent response was significantly attenuated (peak firing 63.2±27.9 impulses/s vs 136.8±30.8 impulses/s in controls, n=9, p≤0.01). Similar reductions were observed in normal animals treated with the TRPV1 antagonist capsazepine for 45min (138.0±41.5 impulses/s vs 167.5±41.5 impulses/s, n=6, p≤0.01). Sensory nerve responses to the nicotinic agonist DMPP were similar in TRPV1 knockout animals and control mice, suggesting the ability of TRPV1 to modulate responses mediated via P2X receptors was specific, and not via a general change in excitability. Contraction responses to αβMe-ATP were unchanged before and after capsazepine incubation, indicating that the interaction occurs at the level of sensory signalling and is not due to changes in detrusor contraction.

Calcium imaging of the dorsal root ganglion neurones innervating the bladder supported this hypothesis, with knockout of the TRPV1 receptor reducing the calcium fluorescent responses (Ratio of F340/380) to 100μM αβMe-ATP from 0.376±0.124 (N=3, n=12) in TRPV1 knockout mice to 0.225±0.075 (N=3, n=15) in wild type control mice. Similar results were obtained when using 1μM ATP responses being reduced from 0.334±0.098 (n=19) to 0.174±0.055 (n=16) in TRPV1 knockout animals.

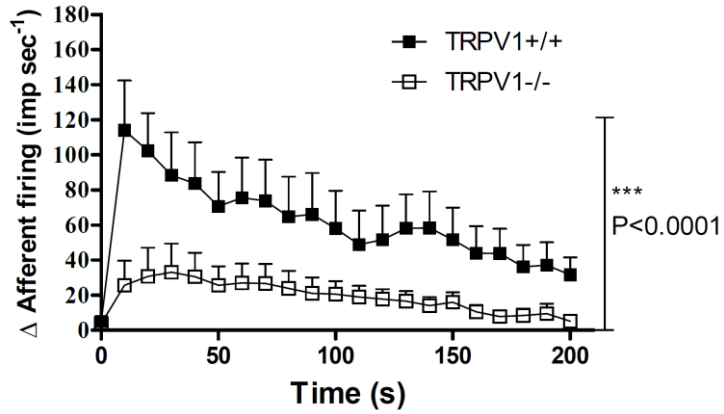

Figure 1: Time course of afferent nerve activity in response to the purinergic agonist αβMe-ATP (30μM). In knockout animals the responses to purinergic receptor stimulation were significantly depressed in TRPV1 knockout animals.

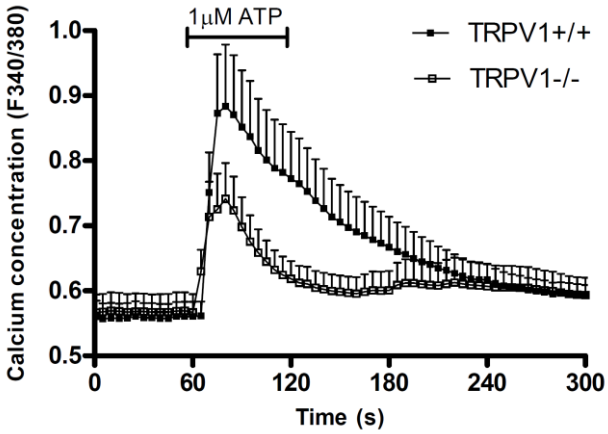

Figure 2: Intracellular calcium responses to ATP (1μM) of dorsal root ganglion neurones from TRPV1 knockout animals and wild type controls.

Interpretation of results

The actions of ATP on bladder sensory nerves is an important component of the micturition reflex. These data demonstrate that these responses are regulated by TRPV1 receptors. Since contractile responses to ATP were not altered by TRPV1 knockout or TRPV1 antagonists, the interaction between the P2X receptor and the TRPV1 receptor must be at the level of the nerve. The interaction is also specific and TRPV1 receptors do not regulate nerve sensitivity to nictotinic receptor stimulation.

Concluding message

These data indicate a significant interaction between P2X and TRPV1 receptors that may be important for pain sensation and micturition. Since the TRPV1 receptor regulated sensory nerve activity, it may provide a novel therapeutic target for the treatment of overactive bladder and interstitial cystitis.

References

1. Vlaskovska, M., et al., P2X3 Knock-Out Mice Reveal a Sensory Role for Urothelially Released ATP. J. Neurosci., 2001. 21(15): p. 5670-5677.
2. Birder, L.A., et al., Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. Nat Neurosci, 2002. 5(9): p. 856-860.
3. Daly, D., et al., Bladder afferent sensitivity in wild-type and TRPV1 knockout mice. The Journal of Physiology, 2007. 583(2): p. 663-674.

Disclosures

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