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CAPSAICIN SENSITIVE AFFERENT NERVE ACTIVITY DURING BLADDER DISTENSION IN NORMAL AND TRPV1 MUTANT MICE.

Abstract Text:

Hypothesis / aims of study

Capsaicin sensitive afferent neurones have been implicated in the neural control of the bladder in many studies. TRPV1 (transient receptor potential vanilloid 1 ion channel) is found on sensory neurons and is the site of action of the vanilloids such as, capsaicin the pungent ingredient of chilli peppers, and structurally related molecules such as resiniferatoxin (RTX) as well as protons and noxious heat (<43^{o}C). Clinically capsaicin and RTX are used to treat functional disturbances in the bladder, however their mechanisms of action and the role of the TRPV1 receptor in pathogenesis of bladder overactivity is still unknown. The aim of the study was to investigate the mechanism of action of capsaicin and RTX in treating overactive bladder and to understand the role of TRPV1 in normal physiology.

Study design, materials and methods

Eight to ten week old male TRPV1 knockout and wildtype mice were used in this study. The whole pelvic section was dissected from the animal and placed in a recording chamber. The chamber was continually superfused with oxygenated Krebs solution at 35^{o}C.The urethra was catheterised using a cannula attached to an infusion pump to enable distension with isotonic saline at an infusion rate of 100µl/min. The dome was catheterised by a two way cannula attached to a pressure transducer and allowing withdrawal of fluid. The catheters were secured in place by ligatures. The pelvic nerve was identified, dissected and placed into a glass suction electrode. The afferent activity was recorded to a computer via a power 1401 interface and Spike 2. All preparations were left to stabilise for 30 minutes before commencing the experiment, after this period repeated distensions to an intravesical pressure of 40mmHg every 10 minutes are carried out until the neuronal activity and pressure response become reproducible. Pharmacological agents were applied either to the superfusate of the bath or intraluminally via the infusion pump and urethral catheter.

Results

The baseline firing in control animals was 0.16 ± 0.04 impulses/s; this was unchanged in the knockout animals. There was no significant difference in compliance between mutants and wildtypes.

To confirm the absence of the TRPV1 channel in the knockouts and to assess the contribution of TRPV1 to normal afferent activity, capsaicin (100nm) and HCl (2M) were intraluminally applied for 3 minutes. Application of capsaicin and HCl in the wild type animals stimulated robust increases in afferent firing. Capsaicin caused a response of 17.54 ± 5.60 % of maximal firing to distension which was attenuated to 0.04 ± 0.20 % in the mutants. Similarly HCl caused a response of 18.56 ± 7.63 % of maximal firing which was attenuated to 1.03 ± 0.5% in the knockout mice.

Gradual bladder distension caused a graded increase in afferent firing in control animals (n=15) with a maximal firing at an intravesical pressure of 40mmHg of 23.54 ± 2.65 imp/sec. This graded afferent response was also observed in the mutants (n=11) but the firing at 40mmHg was significantly reduced (p=0.02, a reduction of roughly 34%) to 13.17±1.08 imp/sec. The influence of TRPV1 on firing was confirmed by the application of the TRPV1 antagonist capsazepine (10µm). Capsazepine produced a reduction in distension induced afferent discharge with mean firing of 16.64 ± 2.93 imp/sec at 40mmHg, a reduction of 36%. Interestingly phasic bladder contractions were seen in 50% of preparations, capsazepine completely abolished this activity.

Spike2 software was used to identify single afferent units from within these whole nerve bundles. Afferent units that responded to distension at intravesical pressure below 15mmHg was classified as low threshold, while fibres with thresholds above 15mmHg was classified as high threshold. The response of these two populations of afferents to capsaicin, capsazepine and distension were analysed. The data shows that 67% of low threshold fibres were capsaicin sensitive, capsaicin increasing afferent discharge by 1174 ± 356 % above baseline (n=19) in these fibres. The distension-induced afferent discharge was again lower in the TRPV1 knockout low threshold fibres than in the wild type low threshold fibres. At an intravesical pressure of 35mmHg mean firing of low threshold fibres from mutant mice was 7.02 ± 0.95 (n=43), a 49% reduction from the wild type low threshold response.

Interpretation of results

These data show that in TRPV1 knockout mice there is no alteration in compliance or in baseline afferent firing. However the studies with both mutants and pharmacological agents showed that the TRPV1 channel is involved in distension induced afferent discharge. Furthermore the data has functionally located the TRPV1 on low threshold afferent fibres thus suggesting that it is involved in normal physiology of the bladder and not just noxious sensation. The demonstration of TRPV1 receptor on low threshold fibres may explain the mechanism by which clinical treatment with capsaicin and RTX may work.

Concluding message

These data suggest that capsaicin or RTX given clinically to treat overactive bladder may act upon the pelvic afferents, desensitising TRPV1 and therefore reducing afferent discharge. This reduction of afferent sensation may delay or suppress the initiation of micturition, reduce the sensation of urgency and depress phasic bladder activity.

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