211

Du S[1], Araki I[1], Nomura T[1], Yoshiyama M[1], Takeda M[1]
1. Department of Urology,Interdisciplinary Graduate School of Medicine and Eng, University of Yamanashi

ACTIVATION OF TRANSIENT RECEPTOR POTENTIAL CHANNEL A1 CAUSES HYPERREFLEXIC MICTURITION BY AFFECTING BLADDER AFFERENT ACTIVITY IN RATS

Hypothesis / aims of study

Transient Receptor Potential channel A1 (TRPA1), which is a non-selective cation channel, emerged as a candidate mechanosensor (1). It shares similar channel properties with mechanotransduction molecules on hair cells. On the other hand, TRPA1 is also a cold receptor, activated from below 17 ºC (2). It is co-localized with TRPV1, CGRP or SP at the small-sized neurons of dorsal root ganglion (DRG) (2), and involved in nociception and heat/cold hyperalgesia (3). These strongly suggest its role in bladder mechanosensory function, bladder cooling reflex or interstitial cystitis. In this study, we examined its expression and role in micturition system of rats.

Study design, materials and methods

Both L_6-S_2 DRG and urinary bladder were taken from Sprague-Dawley female adult rats. RT-PCR and immunohistochemistry (IHC) were performed to demonstrate the expression of TRPA1.

The sheet of bladder mucosa was also separated from muscular layer and subjected to whole mount double immunofluorescence staining with the use of antibodies against TRPA1 and CGRP, in order to stain the submucosal nerves. To further validate its expression at the bladder-innervating primary sensory neurons, the DRG were retrogradely labeled by the injection of a red fluorescent dye, 1,1'-dioctadecyl-6,6'-di(4-sulfophenyl)-3,3,3',3'-tetramethylindocarbocyanine (SP-DilC_{18}(3)), into the bladder wall 2 weeks earlier. The bladder-innervating neurons were confirmed by the red fluorescence and then subjected to another fluorescence staining using primary rabbit anti-TRPA1 antibody, followed by FITC labelled secondary antibody.

Cystometrogram (CMG) was performed, by infusing agonists of TRPA1, *trans*-cinnamaldehyde (CA) or allyl isothiocyanate (AITC), intravesically in normal and capsaicin-treated urethane-anesthetized rats.

Results

RT-PCR showed that TRPA1 mRNA was expressed at L_6 - S_2 DRG, both mucosa and muscular layer of urinary bladder. Some small- to medium-sized neurons in L_6 - S_2 DRG showed TRPA1 immunoreactivity (Fig 1A,B). However, IHC showed only weak staining at smooth muscle but not on mucosa of bladder (Fig 1C). Whole mount double immunofluorescence staining demonstrated the immunoreactivity of TRPA1 on the CGRP-positive sensory nerve terminals in the submucosa of bladder (Fig 1G,H,I). Of DRG neurons innervating the bladder identified by retrograde labeling, 50.8 % showed positive TRPA1 immunoreactivity (Fig 1D,E,F).

Sixty μM CA showed a tendency to decrease pressure threshold (PT) and intercontraction interval (ICI), and 0.6 mM CA decreased the PT, ICI and maximal voiding pressure (MVP) to 83.8%, 59.8% and 89.0% of their control values, respectively (p<0.05, n=5) (Fig 2). The decreases of PT and ICI were reversed to 109.3 and 100.3% of control values respectively (p<0.05), by washing out the drug with saline. Desensitization of C-fiber by capsaicin significantly attenuated the effects of 0.6 mM CA on PT and ICI (n=4) (Fig 3). Similar to CA, 0.4 mM AITC also decreased the ICI (p<0.01, n=4), which was reversible by washing out the drug (p<0.05).

Interpretation of results

We demonstrated that TRPA1 was expressed in around half (50.8 %) of the bladder-innervating primary sensory neurons of DRG. These were mainly small- to medium-sized cells. TRPA1 was also confirmed to co-localize with CGRP in sensory nerve terminals running beneath bladder epithelium, with the use of whole mount double immunofluorescence staining. The weak immunoreactivity of TRPA1 in urinary bladder was probably due to either the relatively lower amount of protein in bladder, or the limited sensitivity of traditional immunohistochemistry. All the morphological data implies that TRPA1 in the DRG might play an important role in the mechanical and/or noxious sensations of the bladder.

A specific antagonist of TRPA1 is unavailable now. Both CA and AITC are specific agonists of TRPA1 (3). The activation of TRPA1 by intravesical infusion of CA resulted in hyperreflexic micturition in a dose-dependent manner. PT and ICI were decreased when activating TRPA1. These effects were reversible when washing out the agonist, so it is less likely to due to inflammation. This hyperreflexia was mediated mainly through C-fibers, because capsaicin pre-treatment attenuated the effects of CA. On the other hand, AITC also decreased the ICI in a reversible way, thus further convincing the role of TRPA1. These functional changes resulting from activation of TRPA1 coincide with the supposed role of a mechanosensor or nociceptor in bladder.

Concluding message

TRPA1 is expressed mainly in the bladder-innervating primary sensory neurons. It may participate in the bladder sensory transduction mechanism, possibly acting as a mechanotransducer and/or nociceptor detecting chemical and thermal stimuli. The clarification of the TRPA1 role in pathological bladder conditions might lead to developing a novel drug target for urinary bladder dysfunction, such as overactive bladder and interstitial cystitis.

References

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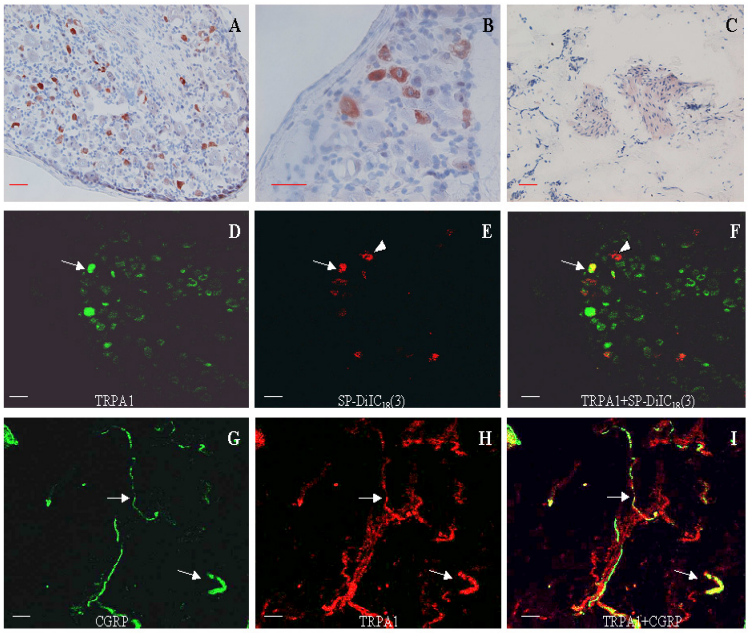

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Fig 1.

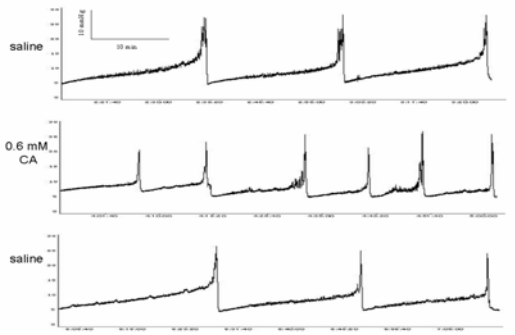

Fig 2.

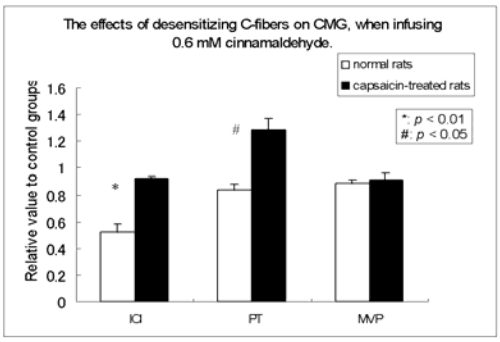

Fig 3.

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