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ALLYL ISOTHYOCYNATE INDUCES BLADDER OVERACTIVITY VIA DIRECT ACTIVATION OF BOTH TRPA1 AND TRPV1

Hypothesis / aims of study

One of the most frequent side effects of cyclophosphamide, a widely used chemotherapeutic agent, is the occurrence hemorrhagic cystitis. These effects are believed to be mediated by acrolein, the metabolite of cyclophosphamide that is excreted in the urine (1). The in vivo effects of electrophilic agents such as acrolein or allyl isothyocynate (AITC) are thought to be due to the activation of TRPA1by covalent binding of cysteine residues (2). TRPA1 and TRPV1 channels are expressed in afferent C-fibres innervating the bladder and are proposed to contribute to the development of bladder overactivity during cystitis (1). To assess the specific role of these receptors, researchers have employed chemical agonists that are believed to be selective for either TRPV1 (capsaicin) or TRPA1 (electrophilic agents such as Allyl isothyocynate, AITC). However, recent evidence indicates that TRPV1 can also be activated by electrophilic agents. Moreover, AITC and capsaicin induce similar effects on the bladder and the urethra (3). Hence, the actual relative role of TRPV1 and TRPA1 in the bladder response to electrophilic compounds is currently unknown. Hypothesis: Electrophilic agents such as AITC can induce bladder overactivity due to direct stimulation of both TRPV1 and TRPA1. Aims: 1) to determine whether mouse TRPV1 is activated by AITC *in vitro* and 2) to perform cystometric measurements in wild type and TRPV1 and TRPA1 knockout mice to investigate the actual contribution of these channels to the bladder response to AITC *in vivo*. This investigation represents a necessary step for establishing valuable experimental models to the study the action of toxic chemotherapeutic agents such as Cyclophosphamide on bladder function.

Study design, materials and methods

Animals: 12 weeks old female wild types, TRPA1$^{-/-}$ and TRPV1$^{-/-}$ mice with a C57BL/6J background were used for in vivo experiments. All animal experiments were carried out in accordance with the European Union Community Council guidelines and were approved by the local ethics committee. *In vivo cystometry:* Intravesical pressure recordings were performed on urethane (1.2 mg/kg) anesthetised animals. Saline was infused at 20 µL/min for 30 min, followed by instillation of AITC dissolved in saline for 30 min. *Ca^{2+} imaging on transfected HEK cells:* Intracellular Ca^{2+} measurements using Fura2 as a Ca^{2+} indicator were performed on Hek293 cells transfected with mTRPV1. *Ca^{2+} imaging on isolated DRG neurons:* Intracellular Ca^{2+} recordings were performed on neurons from L6-S1 dorsal root ganglia (DRG). Neurons were obtained from WT and TRPA1$^{-/-}$ mice.

Results

Ca^{2+} imaging on transfected HEK cells: Application of AITC (5 mM) induced an increase in $[Ca^{2+}]_{in}$ in HEK-293 cells transfected with mouse TRPV1, but not in non-transfected cells. The increase in $[Ca^{2+}]_{in}$ was due to Ca^{2+} influx through TRPV1 channels expressed in the plasma membrane because AITC was ineffective in the absence of extracellular Ca^{2+}. In addition, the increase in $[Ca^{2+}]_{in}$ was strongly reduced by application of 10 µM capsazepine, a TRPV1 antagonist. *Ca^{2+} imaging on isolated DRG neurons:*We also tested whether AITC is able to activate TRPV1 in native chemosensory neurons (mouse DRG neurons). AITC induced Ca^{2+} influxes in DRG neurons from wild type, but also from TRPA1$^{-/-}$ mice. *Cystometry*: In wild type mice intravesical instillation of 500 µM (n = 4) and 1 mM AITC (n = 5) had no statistically significant effect on the intercontractile (ICI) interval, with 5.4 ± 5.8% and 17.4 ± 10.9% reduction of the ICI compared to the baseline value. In contrast, 10 mM AITC induced a strong 66,7 ± 8.7% reduction of the ICI (n = 7, $p < 0.001$). In TRPA1$^{-/-}$, 10 mM AITC also induced bladder overactivity, with a 57.9 ±4.3% reduction of the ICI (n = 6, $p < 0.001$). In TRPV1$^{-/-}$ 10 mM AITC, reduction of the ICI was only 35.7 ± 8.1% (n = 6, $p < 0.05$).

Interpretation of results

Application of AITC to cells heterologously expressing mouse TRPV1 induced a capsazepine-sensitive Ca^{2+} influx. AITC caused a Ca^{2+} influx in native sensory neurons from wild type and TRPA1$^{-/-}$ mice, demonstrating that TRPA1 is not the only AITC receptor in these neurons. These data strongly indicates that AITC can directly activate TRPV1 *in vitro*. Our *in vivo* data show that 10 mM AITC is able to induce bladder overactivity in wild type mice. TRPA1$^{-/-}$ mice are still largely responsive to AITC, suggesting that an important part of the effect of AITC is mediated by another receptor. Notably, the response to AITC in TRPV1$^{-/-}$ mice was significantly smaller than in wild type and TRPA1$^{-/-}$ mice. This strongly indicates that the response to AITC is largely mediated by TRPV1.

Concluding message

Our data demonstrate that AITC activates the capsaicin receptor TRPV1 *in vitro* and *in vivo*. Therefore, contrary to the current believe, bladder overactivity induced by toxic reactive chemical agents can be a consequence of a direct activation of both TRPA1 and TRPV1.

References

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Animal Ethics Committee of KU Leuven**