Application Note

Channel: hTRPA1
Cells: HEK293
Tools: Port-a-Patch

TRPA1 activation by allyl isothiocyanate recorded on the Port-a-Patch

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Cells were kindly provided by EMD Millipore, USA.

Summary

Transient receptor potential (TRP) channels are an important class of receptors found widely distributed throughout the mammalian central and peripheral nervous systems. They have been shown to be activated by many stimuli including temperature, mechano-stimulation, divalent cations and pH, amongst others. TRP channels are receiving much attention as potential targets for the treatment of, for example, pain, respiratory diseases such as asthma, cancer and immune disorders (for review see ref. 1).

The TRPA1 receptor was first cloned from cultured human lung fibroblasts but has subsequently been found to be expressed in sensory neurones and is often found co-localised with TRPV1 (for review see ref. 2). TRPA1 is activated by a number of chemical stimuli including allyl isothiocyanate (mustard oil), cinnamaldehyde (the active ingredient of cinnamon), chlorobenzylidene malononitrile (CS tear gas), hydrogen peroxide and hyperchlorite (chlorine gas). It is thought that TRPA1, together with TRPV1, may contribute to chemical hypersensitivity, chronic cough, and airway inflammation in asthma[2].

Here we present data recorded on the Port-a-Patch® with external perfusion showing recordings of human TRPA1 (hTRPA1) activated by increasing concentrations of allyl isothiocyanate (AITC). hTRPA1 could be repetitively stimulated using low concentrations of AITC but was desensitized by high concentrations of AITC (>10 µM).

Results

Current responses of a cell expressing hTRPA1 and activation by AITC are shown in Figure 1.

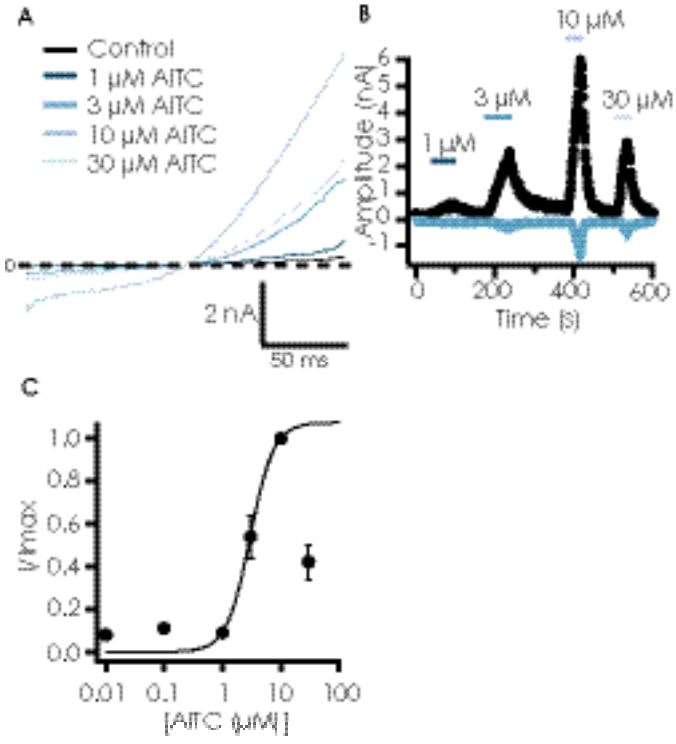

Figure 1:
The TRPA1 current could be activated by increasing concentrations of AITC. **A** Current responses of an exemplar HEK cell stably transfected with hTRPA1 to increasing concentrations of AITC (1 - 30 µM). **B** Time-course of the experiment. Bars indicate application of AITC at the concentrations shown. **C** The concentration response curve for AITC. EC_{50} = 2.7 ± 0.4 µM (n = 5), Hill coefficient = 2.5 ± 0.2 µM (n = 5). The EC_{50} value agrees well with the literature values[2,3].

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hTRPA1 was activated by AITC in a concentration-dependent manner. The maximal response was elicited with 10 µM. 30 µM elicited a smaller response than 10 µM, presumably due to receptor desensitization. The EC_{50} for AITC was calculated from the concentration response curve as 2.7 ± 0.4 µM (n = 5). This is in good agreement with values reported in the literature[2,3].

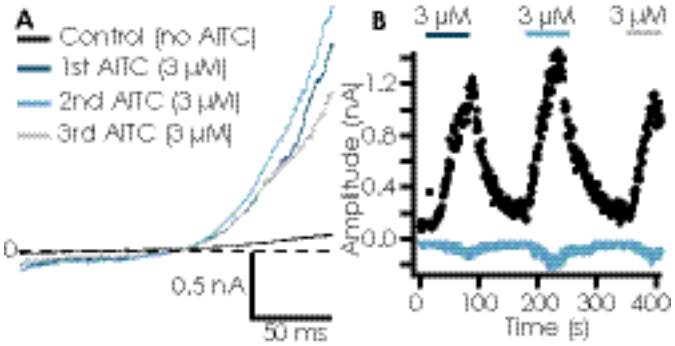

Figure 2:
The hTRPA1 current could be repetitively stimulated by 3 µM AITC. **A** hTRPA1 was activated by 3 consecutive applications of 3 µM AITC. Using this concentration of AITC, TRPA1 could be reliably stimulated. **B** Timecourse for the experiment. Bars indicate application of 3 µM AITC.

Using low concentrations of AITC (3 µM), TRPA1 could be repetitively stimulated in the same cell. Figure 2 shows the current response of hTRPA1 to 3 consecutive applications of 3 µM AITC.

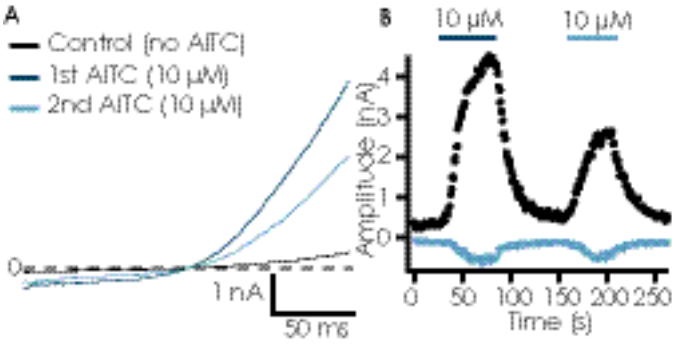

Figure 3:
The hTRPA1 current was desensitized by 10 µM AITC. **A** hTRPA1 was activated by 2 consecutive applications of 10 µM AITC. In this case, the 2nd application of AITC produced about 50% of the current amplitude achieved with the first, presumably due to receptor desensitization. **B** Time-course for the experiment. Bars indicate application of 10 µM AITC.

When a higher concentration of AITC was used (10 µM), the response to the 2nd application of AITC was approximately 50% of that of the first.

In summary, HEK cells stably transfected with the hTRPA1 receptor can be captured to the patch clamp aperture of the Port-a-Patch and TRPA1 can be activated using AITC giving an EC_{50} value consistent with those published in the literature. Using a submaximal concentration of AITC (3 µM), hTRPA1 could be repetitively stimulated making pharmacological experiments possible. Higher concentrations of AITC caused strong desensitization of the channel. The Port-a-Patch provides an excellent tool for studying the TRPA1 channel and it's agonists and antagonists acting both internally and externally.

References

1. Clapham, D., 2003. Nature. 426: 517-524
2. Bessec, B.F., Jordt, S-E., 2008. Physiology. 23: 360-370
3. McNamara C.R., *et al,* 2007. PNAS. 104 (33): 13525 - 13530

Methods

Cells

PrecisION hTRPA1-HEK recombinant cell line (CYL3066) was kindly supplied by EMD Millipore, USA.

Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocol.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Port-a-Patch. The external perfusion system was also used. Currents were recorded every 1 s by 200 ms voltage ramps from -100 mV to +100 mV. Currents were elicited by application of AITC *via* the external perfusion system. AITC was made as a 10 mM stock in DMSO and diluted in external recording solution to the concentrations indicated.

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