B'SYS GmbH

HEK-293 TRPA1 Cell Line

1. Material and Methods

For manual patch-clamping a setup equipped with an EPC-10 amplifier and Patchmaster Software was used. For automated patch-clamping a 16 channel Q-Patch system was used.
Cells were clamped at -80 mV and voltage ramps (-100 to +60 mV, 2 s) were applied every 10 s. Current amplitudes at -100 mV and +60 mV were analyzed, averaged and normalized to control conditions.
The extracellular solution contained (in mM) NaCl 137, KCl 4, CaCl2 1.8, MgCl2 10, Hepes 10, D-Glucose 10. The pH was adjusted to 7.4 with NaOH. The intracellular solution consisted of (in mM) KCl 130, MgCl2 1, Mg-ATP 5, EGTA 5. The pH was adjusted to 7.3 with KOH. After formation of a Gigaohm seal between the patch electrodes and individual TRPA1 stably transfected HEK TRPA1 cells, the cell membrane across the pipette tip was ruptured to assure electrical access to the cell interior. All solutions applied to cells were continuously perfused and maintained at room temperature. As soon as a stable seal could be established the cells were clamped at -80 mV and the voltage stimulus was applied continuously.

2. Agonists

TRPA1 channels were activated with increasing concentrations of Supercinnamaldehyde (0.03 µM – 1.0 µM) or AITC (0.01 µM – 10 µM). Due to desensitisation at high concentrations the EC_{50} of Supercinnamaldehyde could not be determined, for AITC the IC_{50} was calculated to be 2.12 µM.

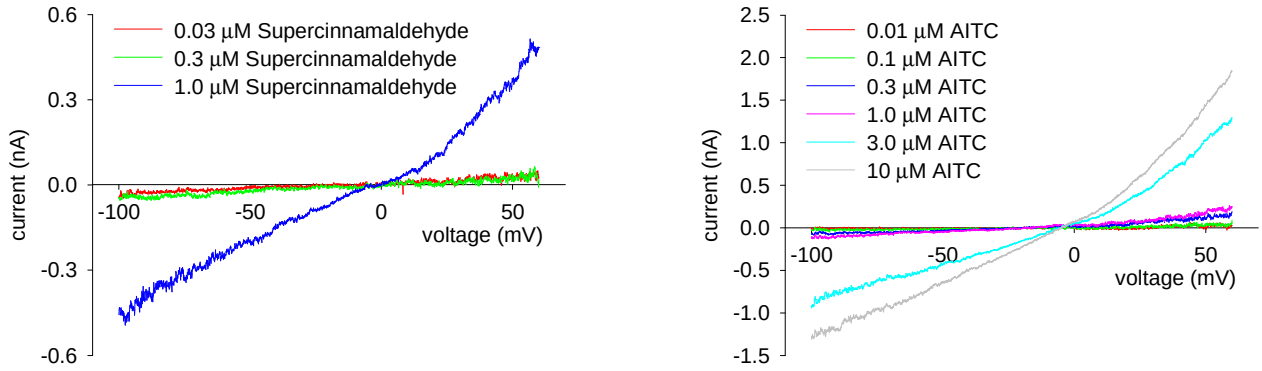

Fig.1: Representative current recordings for HEK TRPA1 cells treated with increasing concentrations of Supercinnamaldehyd or AITC. Currents of voltage ramps at different concentrations were superimposed. Endogenous background current (recorded under control conditions) were subtracted.

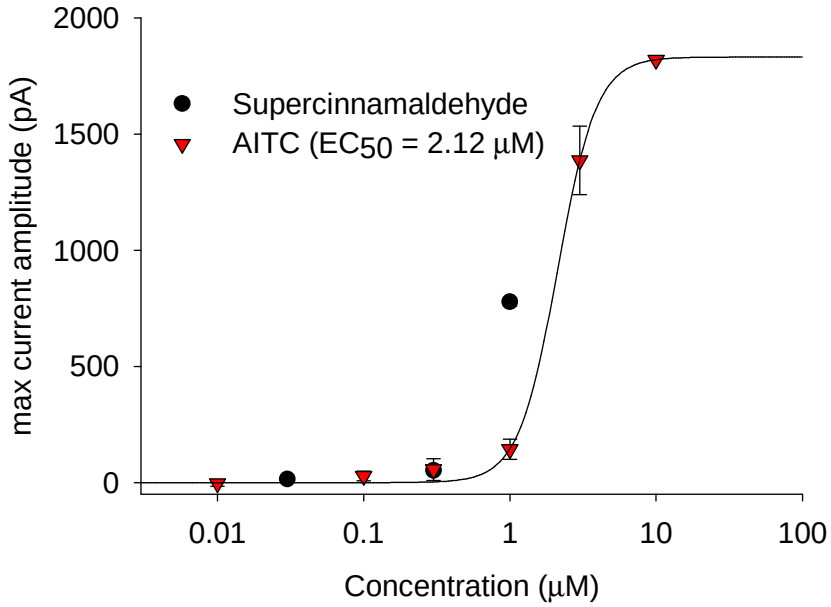

Fig.2: Dose response curves for Supercinnamaldehyde and AITC. Due to desensitisation of currents at concentrations higher than 1 µM of Supercinnamaldehyd, no EC_{50} could be determined. (current amplitudes at the maximal current amplitude were averaged for at least n=2 cells and plot versus the perfused concentration of agonist. Data were fit with a 3 parameter logistic equation)

3. Antagonists

The unselective TRP antagonist Ruthenium Red was used to test the block of TRPA1 channels stimulated with 1 µM Supercinnamaldehyde. The maximal current amplitude of each voltage ramp was normalized to the maximal current amplitude under control conditions. The IC_{50} of Ruthenium Red was determined to be 12.25 nM.

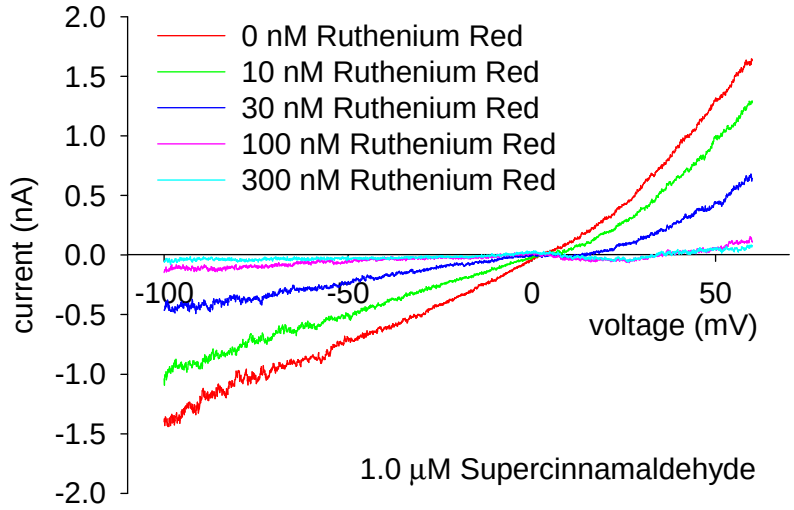

Fig.3: Representative current recordings for HEK TRPA1 cells treated with increasing concentrations of Ruthenium Red in the presence of 1 µM Supercinnamaldehyd. Currents of voltage ramps at different concentrations were superimposed. Endogenous background current (recorded under control conditions) were subtracted.

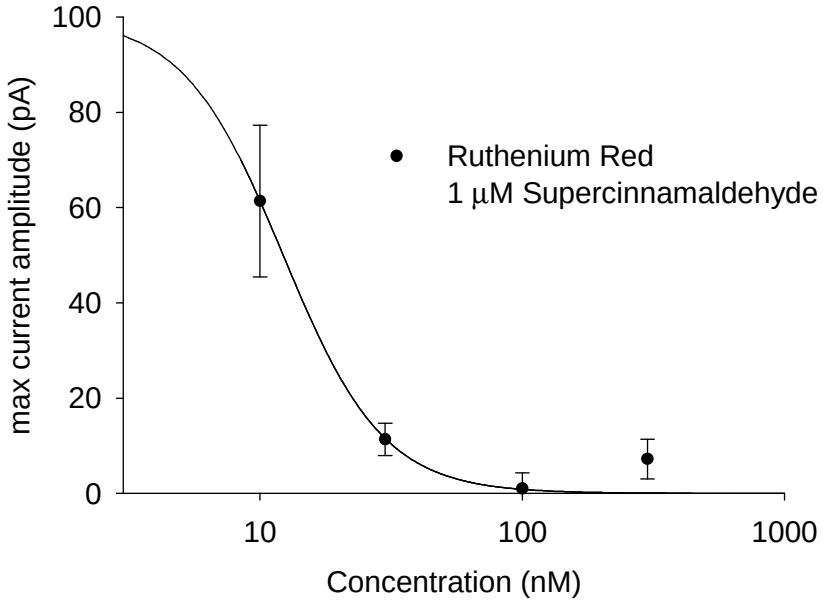

Fig.4: Dose response curve for Ruthenium Red at the presence of 1 µM Supercinnamaldehyde (current amplitudes at the maximal current amplitude were averaged for at least n=2 cells and plot versus the perfused concentration of Ruthenium Red. Data were fit with a 3 parameter logistic equation)