

Reproducible activation and pharmacology of hTRPA1 on the SyncroPatch 384

The electrophysiology team at Nanion Technologies GmbH, Munich. Cells were kindly supplied by Charles River.



Summary

The transient receptor potential ankyrin 1 (TRPA1) is a calcium permeable non-selective cation channel that belongs to the transient receptor potential (TRP) superfamily. The TRPA1 channel is expressed in the sensory neurons of the nodose ganglia, dorsal root ganglia, and trigeminal ganglia¹⁻³, and also non-neuronal cells such as cardiomyocytes, lung fibroblasts and pancreatic β cells^{1,3}. TRPA1 is activated by a range of natural pungent compounds including allyl isothiocyanate (AITC), cinnamaldehyde, and allicin⁴⁻⁶. TRPA1 can also be activated by cold temperature^{3,7} and has been proposed to act as a mechanosensor³. Not only has TRPA1 been proposed to play a role in nociception and certain pain conditions⁸⁻¹⁰, but has also in cardiovascular conditions such as atherosclerosis, heart failure, arrhythmia, vasodilation, and hypertension¹. Thus, within drug development, much attention is paid to the TRPA1 channel. For example, TRPA1 has been identified as a potential target for persistent chronic painful states including inflammation, neuropathic pain, diabetes, fibromyalgia, bronchitis, and emphysema¹¹. Indeed, the TRPA1 antagonist GR 17536 from Glenmark showed efficacy in a Phase IIa proof-of-concept clinical trial for peripheral diabetic neuropathy¹¹.

The most challenging aspects involved in the screening of the TRPA1 channel are the channel's mechanosensitivity, fast desensitization and activity dependence on intracellular calcium. Here, we present high quality data with reliable pharmacology on hTRPA1 expressing CHO cells collected on the SyncroPatch 384. Data is presented showing activation of the TRPA1 channel by carvacrol by the antagonists AMG0902 and A967079.

Results

Human TRPA1 expressed in CHO cells was activated by carvacrol in a concentration-dependent manner (Fig. 1). Carvacrol, a major component of oregano, has been shown to activate TRPV3 and TRPA1 channels¹². TRPA1 currents activated by carvacrol quickly desensitize in the continued presence of the agonist and repeated activation results in smaller responses with subsequent addition of the same concentration of ligand (tachyphylaxis)¹².

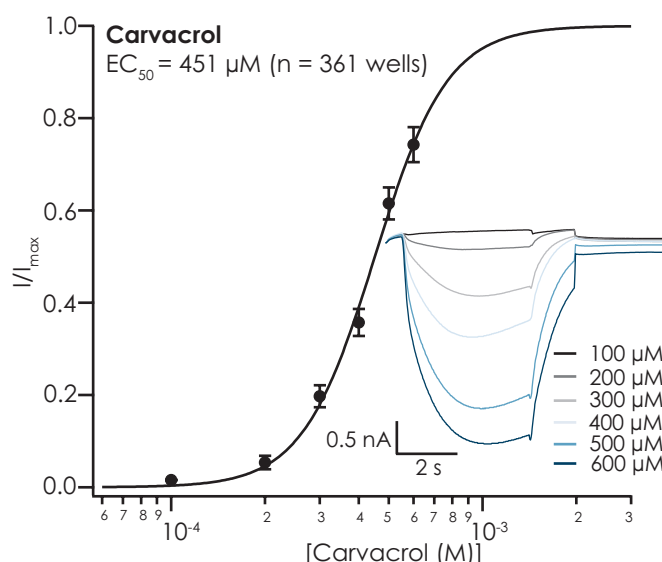


Figure 1: Activation of TRPA1 by carvacrol recorded on the SyncroPatch 384. The concentration response curve for carvacrol for an average of 361 wells is shown with the average traces for each concentration shown in the inset. Multihole chips (4 holes per well) were used. TRPA1 was recorded using a constant holding potential of -60 mV, a single concentration of carvacrol was added to each well and the concentration response curve calculated across multiple wells.

Application Note

In order to minimize desensitization of the channel and allow repetitive activation of TRPA1, the LigandPuff approach of the SyncroPatch 384 was used for the experiments. A small amount of ligand was used to activate TRPA1 with a short wait time of 4 secs to ensure full activation of the current but minimizes desensitization. Using this approach, TRPA1 could be repetitively activated by repeated stimulation with 300 μ M carvacrol (Fig. 2) allowing inhibitors to be studied.

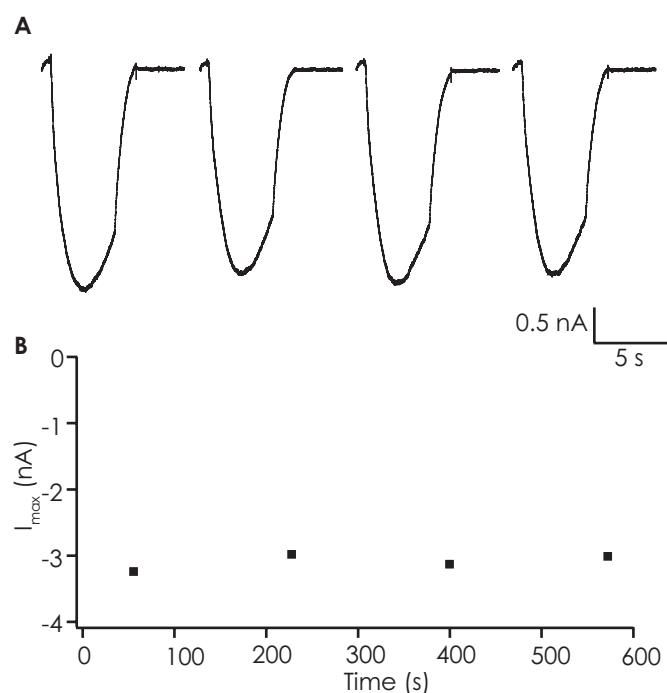


Figure 2: Repetitive activation of TRPA1 by carvacrol. Using the LigandPuff technique, TRPA1 could be repetitively activated by 300 μ M carvacrol at least 4 times in the same well with a peak amplitude consistent with prior applications (<10% change in peak amplitude). Shown are raw current traces in response to 300 μ M carvacrol from an exemplar cell in (A) and the corresponding time course of the experiment in (B).

Each well received 3 x applications of 300 μ M carvacrol followed by co-application of carvacrol and a single concentration of either AMG0902 or A967079. Figure 3 shows the concentration response curve for AMG0902 for an average of 337 wells. The IC_{50} for AMG0902 was calculated to be 35.9 nM in good agreement with the literature value of 68 ± 38 nM for AITC activation of rTRPA1 in a Ca^{2+} imaging assay¹³.

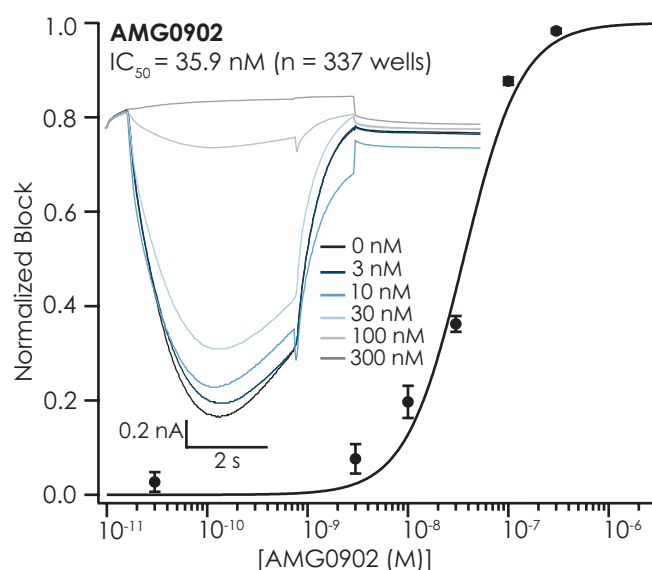


Figure 3: Average concentration response curve for AMG0902. A single concentration of AMG0902 was co-applied with 300 μ M carvacrol and the concentration response curve for an average of 337 wells is shown. Average traces in different concentrations of AMG0902 are also shown in the inset. The IC_{50} for AMG0902 was 35.9 nM ($n = 337$) giving a success rate of 88% for completed experiments.

A967079 is a selective inhibitor of TRPA1 with an IC_{50} in the nM range and does not block TRPV1, TRPV2, TRPV3, TRPV4 or TRPM8 channels¹⁴. In our experiments, hTRPA1 activated by 300 μ M carvacrol was blocked by A967079 with an $IC_{50} = 9.8$ nM ($n = 331$ wells; Fig. 4), in good agreement with the literature value of 51 nM for hTRPA1 activated by 100 μ M AITC using manual patch clamp¹⁴.

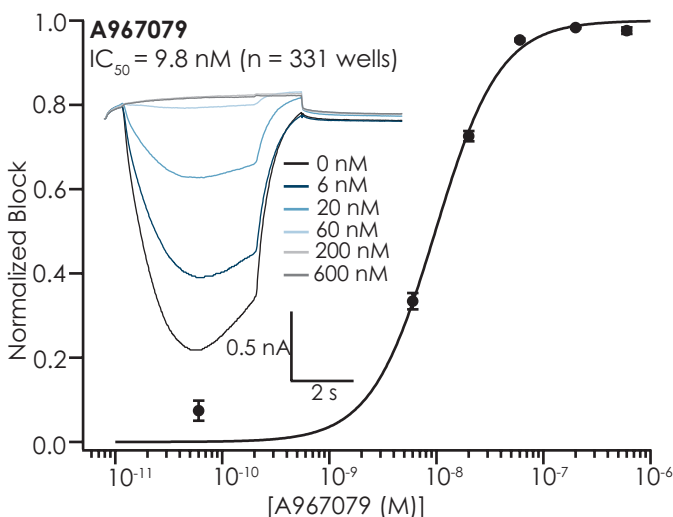
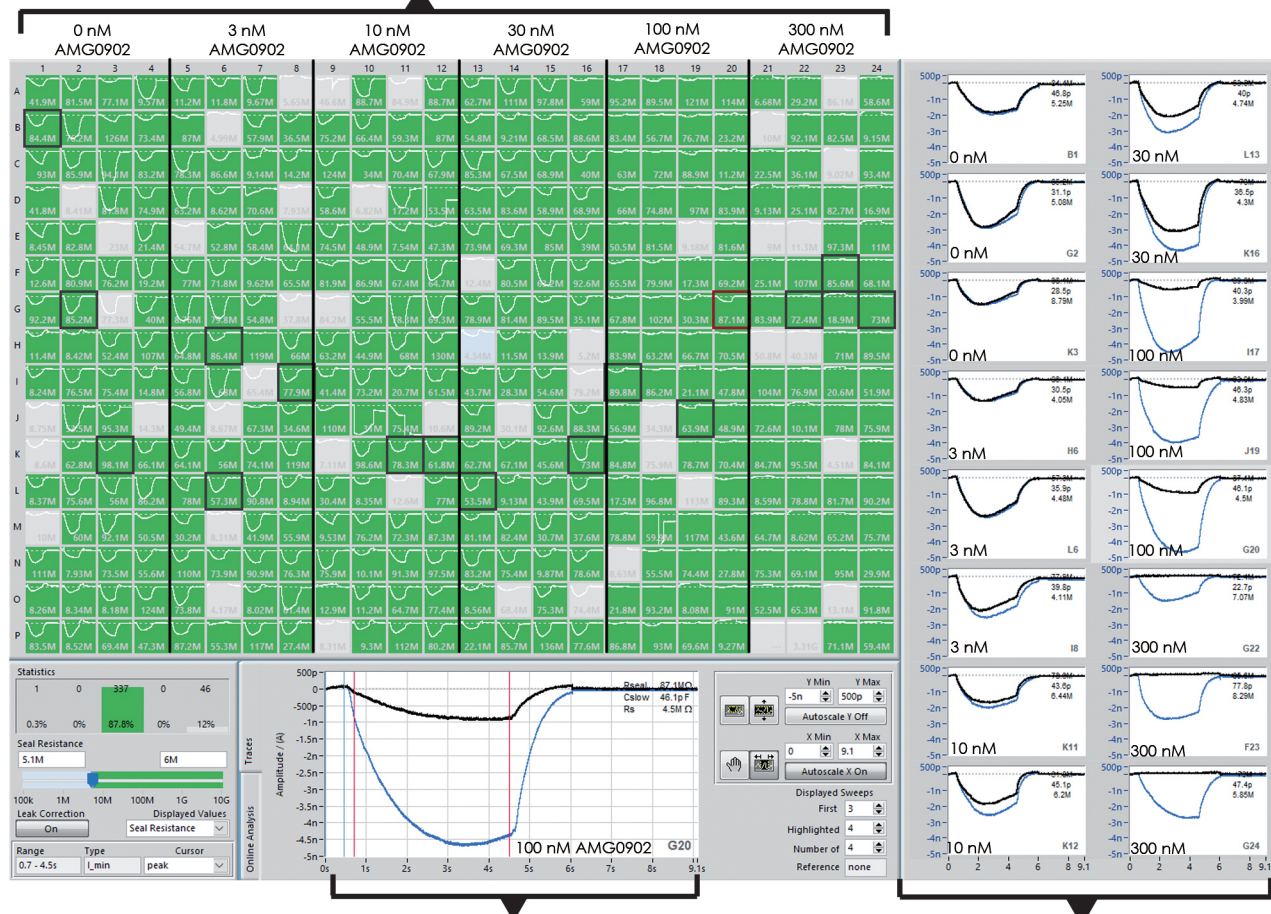


Figure 4: Average concentration response curve for A967079. A single concentration of A967079 was co-applied with 300 μ M carvacrol and the concentration response curve for an average of 331 wells is shown. Average traces in different concentrations of A967079 are also shown in the inset. The IC_{50} for A967079 was 9.8 nM ($n = 331$) giving a success rate of 86% for completed experiments.

Application Note

384 color coded depictions of data traces eases judgement of success rate



Highlighted raw traces of 1 cell showing control amplitude and effect of 100 nM AMG0902 on TRPA1-mediated currents

Highlighted raw traces of 16 cells showing inhibition of TRPA1 by AMG0902

Figure 5: Graphical user interface of the screening and data analysis software used on the SyncroPatch 384. Screenshot of depiction of raw current traces of TRPA1 expressed in CHO cells as recorded on one NPC-384 patch clamp chip (4x holes per well). Three hundred and eighty-four small color-coded pictures as seen in the upper left part display 384 recordings. Wells used for analysis are shown in green and grey wells indicate failed wells based on the quality control criteria. One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. Graphs show current traces of hTRPA1 after activation with 300 μ M carvacrol (blue trace) and with co-application with AMG0902 at the given concentrations (black trace).

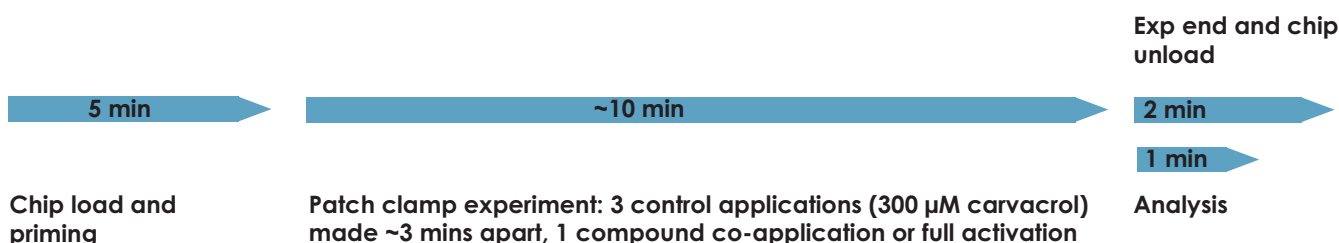


Figure 6: The completion of 1 experiment on the SyncroPatch 384 patch clamp chip (384 wells) for a single point concentration response curve plus activation of TRPA1 with carvacrol took approximately 17 min.

Application Note

Figure 5 shows a screenshot of the SyncroPatch 384 software during an experiment. A color-coded overview (based on seal resistance in this case) of all 384 wells gives the user a good impression of the success rate of the experiment. The user can choose whether to visualize raw traces or online analysis. In this case, raw current traces are shown, blue traces indicate control (300 μ M carvacrol) and black traces in the presence of AMG0902. An individual well can be highlighted to monitor the progression of the experiment.

In conclusion, hTRPA1 expressed in CHO cells can be recorded on the SyncroPatch 384 with a good success rate for completed experiments (>86%). The timeline of each experiment was about 17 minutes (start – end) and included activation with carvacrol (repeated 3 times

to ensure stability) and application of a single compound concentration to each well.

Using the LigandPuff application procedure of the SyncroPatch 384, TRPA1 could be repetitively activated. As expected, hTRPA1 could be activated by carvacrol¹¹ and inhibited by AMG0902 and A967079 in a concentration-dependent manner with IC_{50} values similar to those reported in the literature^{13,14}.

The SyncroPatch 384 is a high throughput and highly reliable automated patch clamp device for recording hTRPA1 currents. User-friendly software, excellent success rates, single additions or multiple additions of compound to each cell and easy analysis result in reliable high quality data at an increased throughput with an economical cost per data point.

References

1. Wang Z., *et al.* 2019. *Front. Pharmacol.* 10: 1253.
2. Story G.M., *et al.* 2003. *Cell.* 112(6): 819-29.
3. Nilius B., *et al.* 2012. *Pflügers Arch. - Eur. J. Physiol.* 464:425–458.
4. Bandell M., *et al.* 2004. *Neuron.* 41 (6): 849-57.
5. Jordt S.E., *et al.* 2004. *Nature.* 427: 260-265.
6. Macpherson L.J., *et al.* 2005. *Curr. Biol.* 15: 929–934.
7. Kwan K.Y., & Corey D.P. 2009. *JGP.* 133(3): 251–256.
8. Kremeyer, B., *et al.* 2010. *Neuron.* 66: 671-680.
9. Koivisto, A., *et al.* 2018. *Pharmaceuticals* 11: 117; doi:10.3390/ph11040117.
10. Hiyama H., *et al.* 2018. *Mol. Pain.* 14: 1744806918789812.
11. Skerratt S., 2017. *Prog. Med. Chem.* 6: 81-115.
12. Xu H., *et al.* 2006. *Nat. Neurosci.* 9(5):628-635.
13. Lehto, S.G., *et al.*, 2016. *Mol. Pain.* 12: doi: 10.1177/1744806916677761.
14. Chen, J., *et al.* 2011. *Pain.* 152(5): 1165-1172

Methods

Cells

hTRPA1 expressing CHO cells were supplied by Charles River, USA.

Cell culture

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

Electrophysiology

Perforated patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch 384 using escin in the intracellular solution. A constant holding potential of -60 mV was used and a small volume of ligand (5 μ l) was applied to activate TRPA1. Multihole chips were used with 4 holes per well. For pharmacology experiments the inhibitor was co-applied with activator after 3 control applications of ligand and data normalized to peak amplitude in the absence of the blocker. A single concentration of blocker was applied to each well and the concentration response curve calculated across multiple wells.