SOPHION BIOSCIENCE

Application Report

HEK293-TRPV1 tested on QPatch

The TRPV1 experiments presented stable current with both single- and multi-hole technology

Summary

Performing experiments on TRP channels can be challenging. However, TRPV1 may be one of the easiest within the TRP-family to perform experiments on and here we tested TRPV1 on the QPatch with single- and multi-hole technology. The results show stable current and good agonist and antagonist experiments.

Introduction

The first mammalian TRPV was identified when searching for channels activated by the inflammatory vanilloid compound capsaicin. TRPV1 are activated by heat and by a range of chemicals including endocannabinoid, anandamide, camphor, garlic and black pepper. TRPV1 conducts cation influx (1).

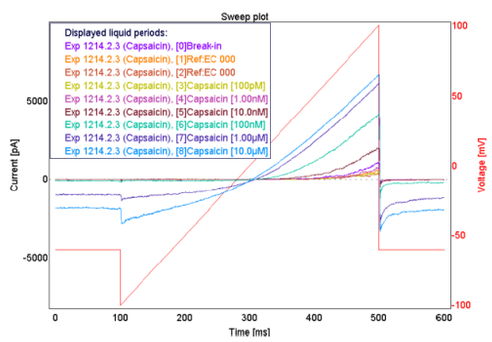
Performing experiments on TRP channels can be challenging. However, TRPV1 may be one of the easiest within the TRP-family to perform experiments on. Hence they are easy to activate and show stable current vs. time behaviour.

Results

Results for single-hole mode

One agonist (capsaicin) and one antagonist (capsazepine) were tested in single-hole mode, as well as in multi-hole mode. Data from the single-hole mode experiments can be seen in Figure 1.

A.



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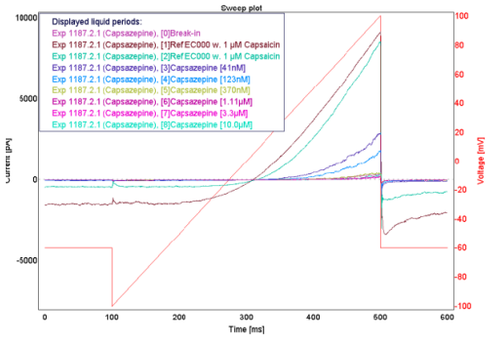
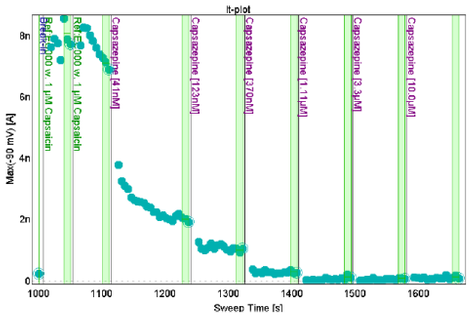


C.



D.

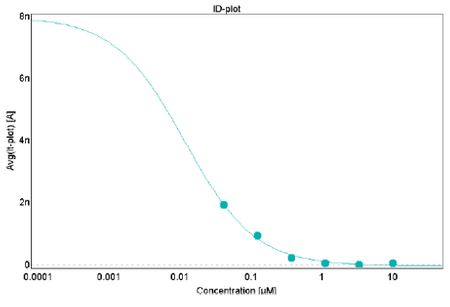
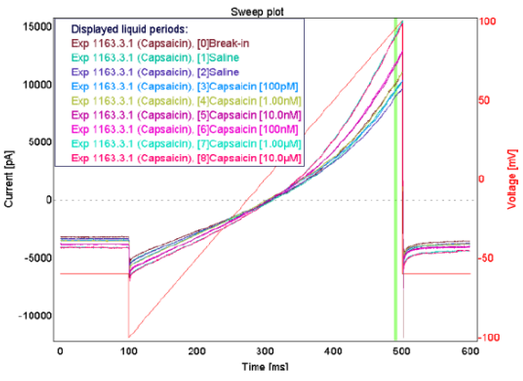


Fig. 1. HEK293-TRPV1 cells in single-hole mode. a) raw current with increasing concentration of capsaicin. b) raw current traces with was activated by 1 µM capsaicin and blocked by increasing concentration of capsazepine. c) TRPV1 current plotted against time, where current is activated by capsaicin and blocked by increasing concentration of capsazepine. d) Hill plot with increasing concentration of capsazepine (IC50=30.7±7.77 nM n=4).

Results for single-hole mode

A.



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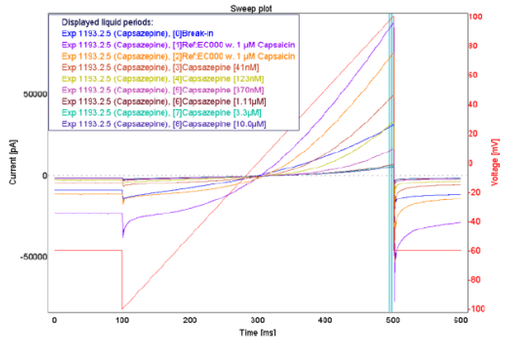

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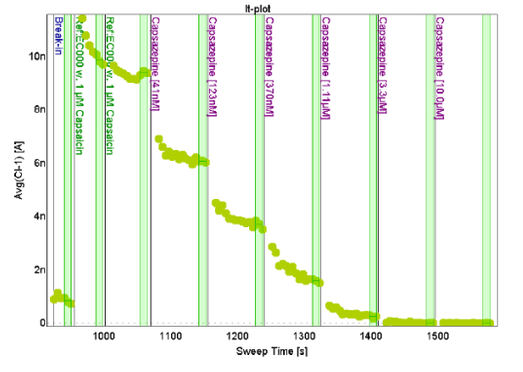

D.

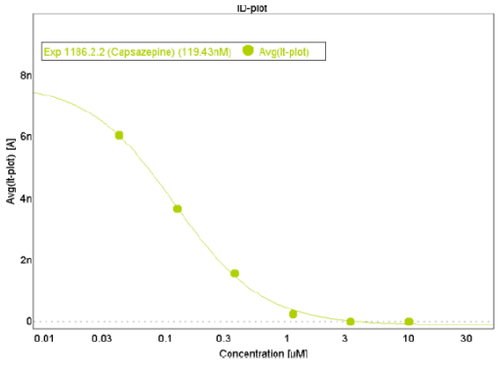

Fig. 2. HEK293-TRPV1 cells in multi-hole mode. a) raw current with increasing concentration of capsaicin (EC_{50}=7.59±6.15 nM n=6, fit not shown). b) raw current traces with was activated by 1 µM capsaicin and blocked by increasing concentration of capsazepine. c) TRPV1 current plotted against time, where current is activated by capsaicin and blocked by increasing concentration of capsazepine. d) Hill plot with increasing concentration of capsazepine (IC_{50}=43.3±12.6 nM n=8).

Performing experiments in multi-hole mode give rise to a higher current level and are useful with low expressing systems. In this study low current level was not a problem and the current level measured at +90 mV with 1 µM capsaicin was 6.7±0.9 nA in single-hole mode and 76.4±2.3 nA in multi-hole mode (Figure 2).

Conclusion

TRPV1 are stable on the QPatch platform and it is easy to perform agonist and antagonist studies on this system. The half maximal effective and inhibitory concentrations (EC_{50} and IC_{50}) found in this study are comparable to previously reported values.

Methods

Cells were cultured and harvested according to Sophion standard operation procedures. The cells used in this study were a stable inducible HEK293 cell line expressing TRPV1.

Whole cell protocol; the same whole cell protocol was used both in single-hole and multi-hole mode. This protocol is a modified timed whole cell protocol.

Voltage protocol; a 400 ms ramp from -100 to +100 mV from a holding potential at -60 mV (see red line in figure 1 & 2). Voltage protocol executed at 0.2 Hz. Data were sampled at 5 kHz and filtered at 1 kHz.

Application protocol; The two test compounds, capsaicin and capsazepine, were each tested at 6 concentrations added as accumulated dosages (see figure 1 & 2). In order to mimic constant perfusion, each test concentration was added four times to each well.

References:

1. Venkatachalam K, Montell C. TRP Channels. Annu Rev Biochem. 2007;76:387-417.

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