Application Report:

HEK293-TRPV1

QPatch

AR_PUBLIC16620-2

Sophion Bioscience A/S, Baltorpvej 154, DK-2750 Ballerup, Danmark
Phone: + 45 44 60 88 00 Fax: +45 44 60 88 99 E-mail: info@sophion.dk www.sophion.com

sophion

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 (Venkatachalam 2007).

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 behavior.

Materials and Methods

Cells; 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.

Ringer's solutions; Intra cellular; (in mM) $CaCl_2$ 5.374, $MgCl_2$ 1.75, KOH 31.25, EGTA 10, HEPES 10, KCl 120, Na_2-ATP 4. pH=7.2, 270-295 mOsm/l. Extra cellular; (in mM) $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, KCl 4, NaCl 145, Glucose 10. pH=7.4, 285-295 mOsm/l.

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 was sampled at 5 kHz and filtered at 1 kHz.

Chemicals; all compounds was ordered from Sigma. Capsaicin (Sigma M2028), Capsazepine (Sigma C191).

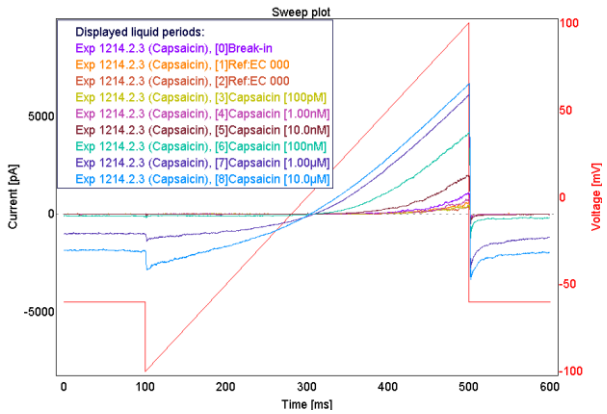
Application protocol; The two test compounds, Capsaicin and Capsazepine, were each tested at 6 concentrations added as accumulated dosages (see figure 1 & 2). In order to mimic constant perfusion each test concentration was added four times to each well.

Results

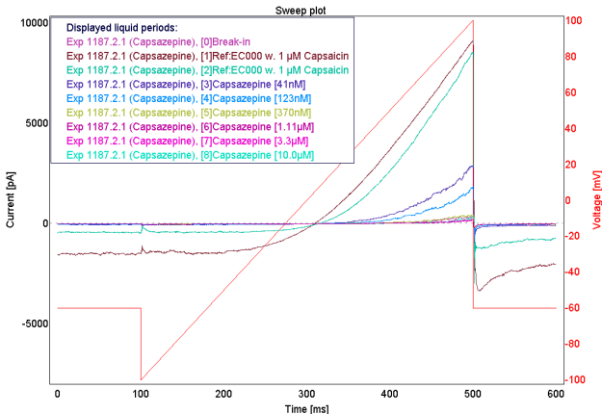
Results for single-hole mode

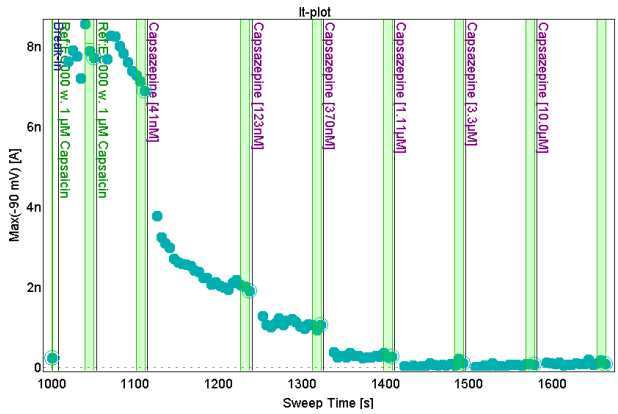
One agonist (Capsaicin) and one antagonist (Capsazepine) were tested in single-hole mode, as well as in multi-hole mode. Data from the single-hole mode experiments can be seen in Figure 1.

A



B





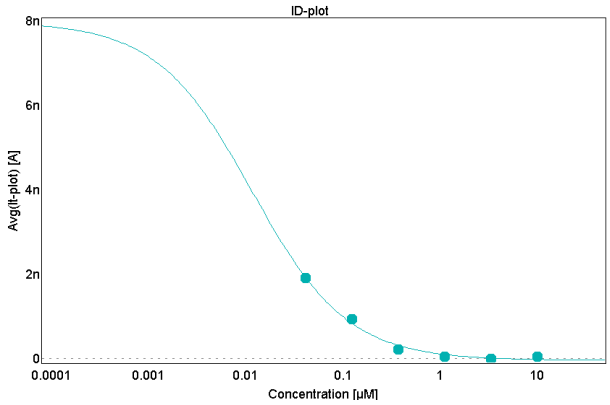
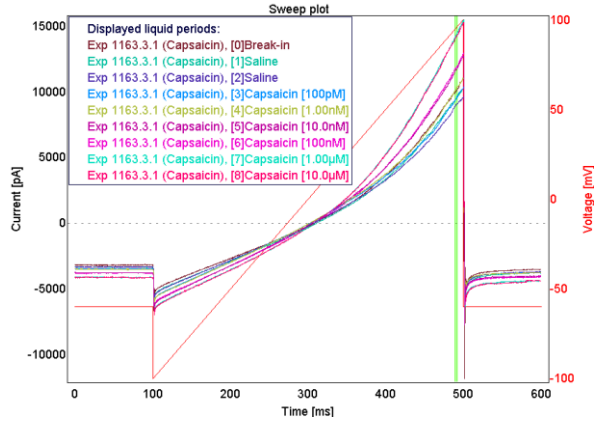


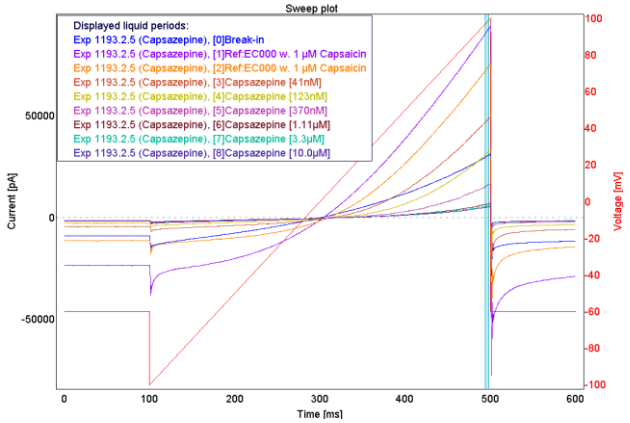
Figure 1. HEK293-TRPV1 cells in single-hole mode. a) raw current with increasing concentration of Capsaicin. b) raw current traces with was activated by 1 µM Capsaicin and blocked by increasing concentration of Capsazepine. c) TRPV1 current plotted against time, where current are activated by capsaicin and blocked by increasing concentration of Capsazepine. d) Hill plot with increasing concentration of Capsazepine (IC_{50}=30.7±7.77 nM n=4).

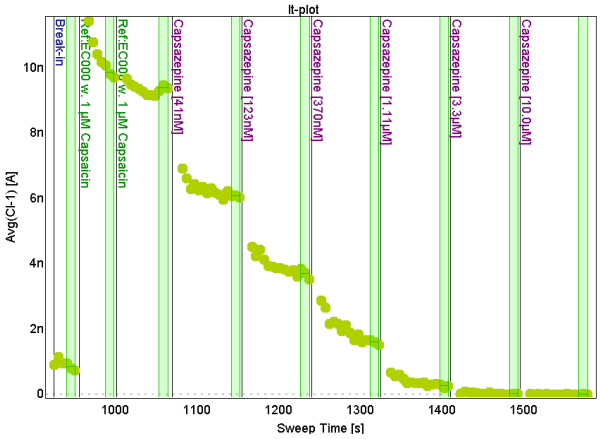
Results for multi-hole mode

a



B





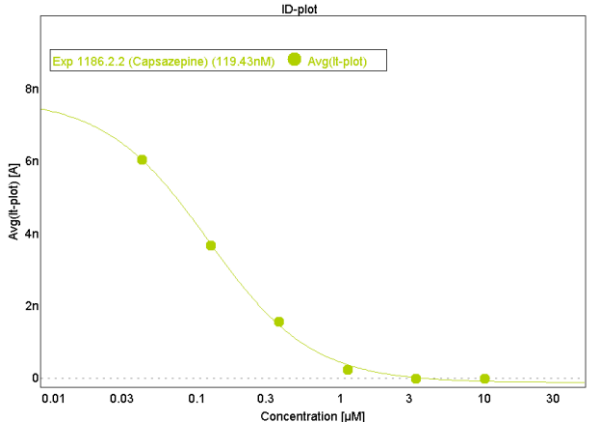


Figure 2. HEK293-TRPV1 cells in multi-hole mode. a) raw current with increasing concentration of Capsaicin (EC_{50}=7.59±6.15 nM n=6, fit not shown). b) raw current traces with was activated by 1 µM Capsaicin and blocked by increasing concentration of Capsazepine. c) TRPV1 current plotted against time, where current are 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 gives rise to higher current level and are particular 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).

References

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

Conclusion

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