SOPHION BIOSCIENCE

Application Report

CHO & HEK-TRPM8 tested on QPatch

Pharmacological characteristics of two TRPM8 expressing cell lines by using multi-hole and single-hole technology on QPatch

Summary

CHO-TRPM8 and HEK-hTRPM8 cells were tested on QPatch which both provided high success rates, high-quality gigaseals and good recordings of the ion channel current. The EC_{50} and IC_{50} estimations for menthol, icilin and capsazepine were in agreement with reported literature values.

Introduction

TRPM8 belongs to the melastation family of transient receptor potential channels. The channel is cold - sensitive and non-selectively conducts mono- and di-valent cations.

The channel is involved in physiological functions including sensing of unpleasant cold, pain and thermoregulation but was also found to be upregulated in pathophysiologies like cancer.

In the present work TRPM8 recombinantly expressed in Chinese hamster ovary (CHO) and HEK293 cells were characterized using QPatch.

Results

The IV relationship of the TRPM8 channel is shown in Figure 1. As expected, the channel exhibits strong outward rectification when activated with menthol (racemic menthol was used in Figure 1). This is in good accordance with what has been presented in the literature (1-4).

Furthermore, application of racemic menthol generated a small inward current at negative potentials (Figure 1, insert). This inward current was a lot more pronounced when using –(-)-menthol (data not shown).

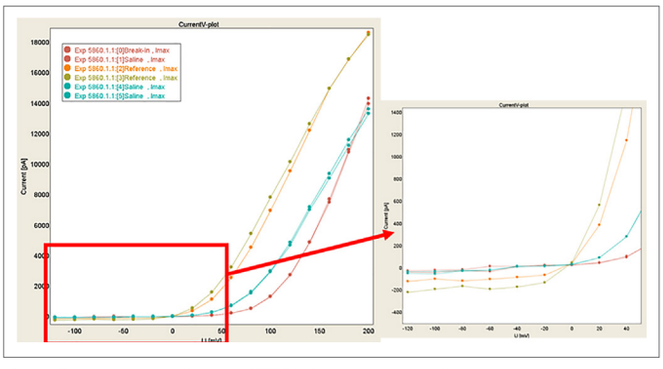


Fig. 1. Representative IV plot of TRPM8 response to saline and racemic menthol ('reference'). An enlargement of the response at negative potentials is shown to the right.

Racemic menthol

Concentration-response relationships of racemic menthol were recorded using a voltage ramp protocol.

Figure 2, 3 and 4 show representative raw traces, IT plot and Hill fit, respectively. Interestingly, racemic menthol did not elicit much current at negative potentials.

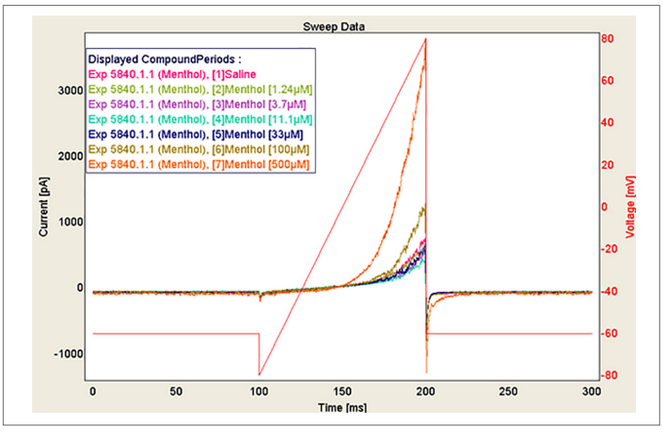


Fig. 2. Representative raw current traces of the response elicited by a voltage ramp from -80 to +80 mV, when subjected to increasing concentrations of racemic menthol as indicated.

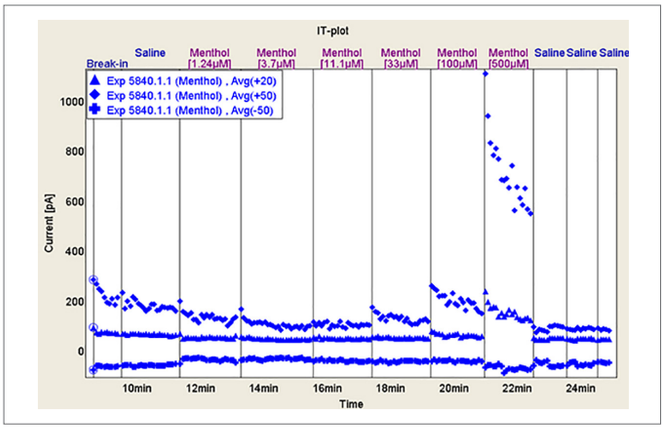


Fig. 3. Representative IT-plot of showing current values at +50 mV (◊), +20 mV (Δ) and -50 mV (+), in response to increasing concentrations of racemic menthol.

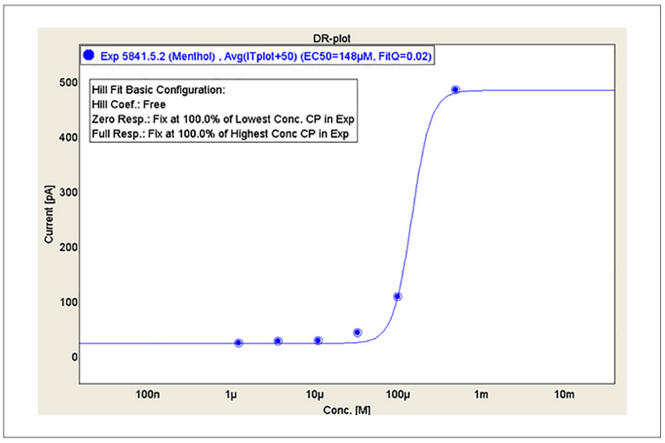


Fig. 4. Representative Hill fit of the concentration-response relationship to racemic menthol (at +50 mV).

Figure 5 summarizes the EC_{50} values at various potentials. No voltage-dependence of menthol stimulation was detected.

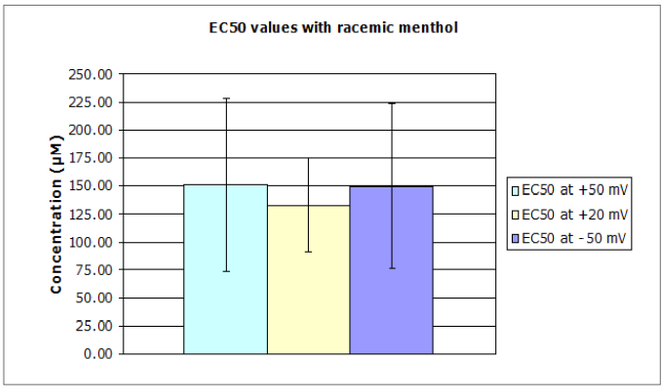


Fig. 5. Summary of EC_{50} ± SD values of racemic menthol at three different potentials: +50 mV, +20 mV and -50 mV (n=30).

(-)-Menthol

In a next step, the effect of (-)-menthol on TRPM8 was tested. Figure 6 shows a representative IT plot of a concentration - response experiment with the (-)-menthol. Exposure to (-)-menthol elicited a more prominent inward current at negative potentials compared with the racemic analogue.

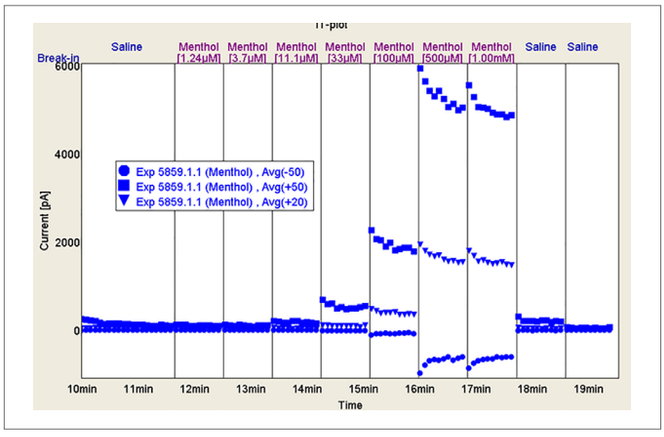


Fig. 6. Representative IT-plot of the current elicited during the ramp (shown in Figure 2) at +50 mV (□), +20 mV (Δ) and -50 mV (O), in response to increasing concentrations of (-)-menthol.

Representative concentration-response curves at three different potentials can be seen in Figure 7, and an overview of the resulting EC_{50} estimations is shown in Figure 8. The obtained EC_{50} is in good agreement with what has been described in the literature (5).

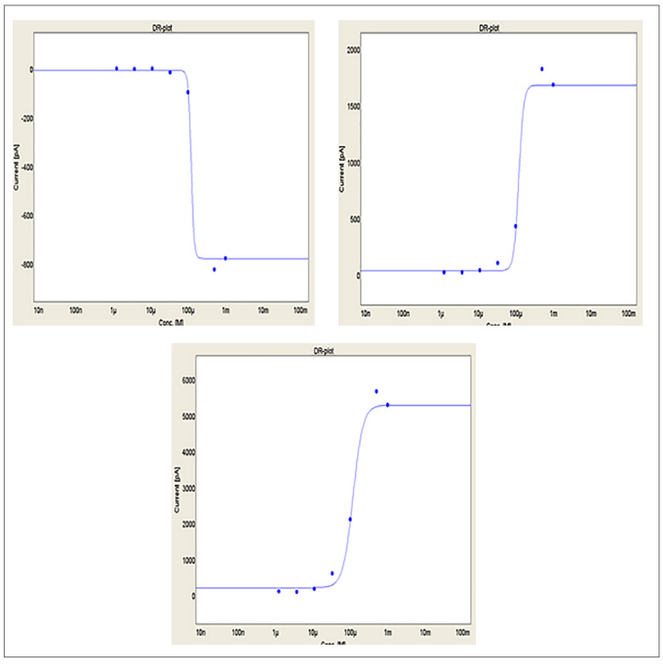


Fig. 7. Representative Hill fit of the concentration - response relationship to (-)-menthol. From the left: current level at -50 mV, +20 mV and +50 mV, respectively.

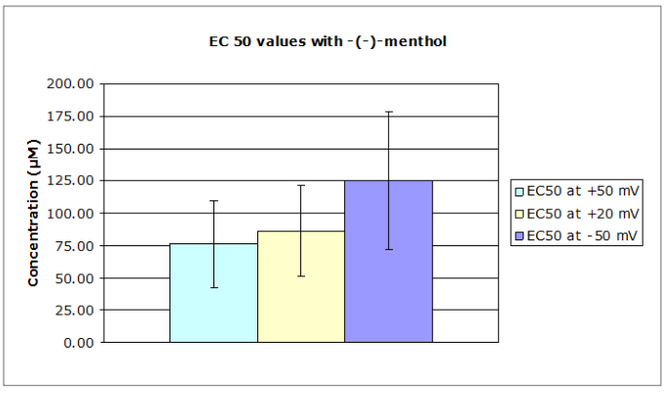


Fig. 8. Average EC_{50} ± SD values obtained with (-)-menthol at three different potentials: +50 mV, +20 mV and -50 mV (n=22).

Icilin Response

Obtaining a response to icilin with TRPM8 can be challenging, because it requires very strict control of free intracellular Ca^{2+} (6). Further complicating is the fact that TRPM8 exhibits a strong Ca^{2+}-dependent desensitization, which obscures the response to icilin (6). Addition of BAPTA to the internal solution can help stabilizing the desensitization.

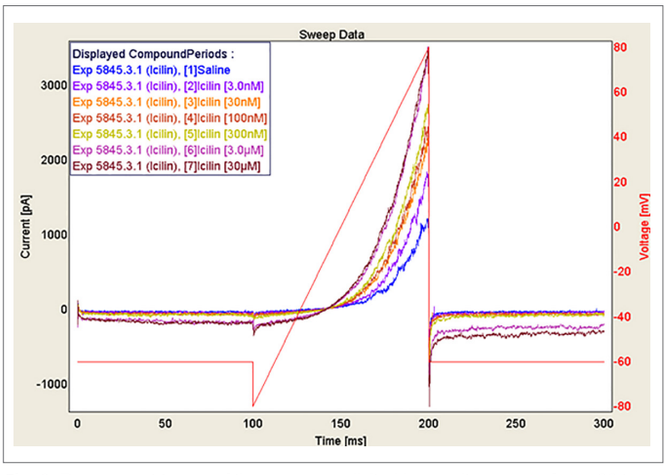


Fig. 9. Raw current traces of the response elicited by a voltage ramp from -80 to +80 mV, when subjected to increasing concentrations of icilin.

Figure 9, 10 and 11 show the raw data, IT plot and Hill fit, respectively, for the icilin response. The EC_{50} value was in this case estimated to be 70 nM, which is in line with literature values of ~ 100 nM (5).

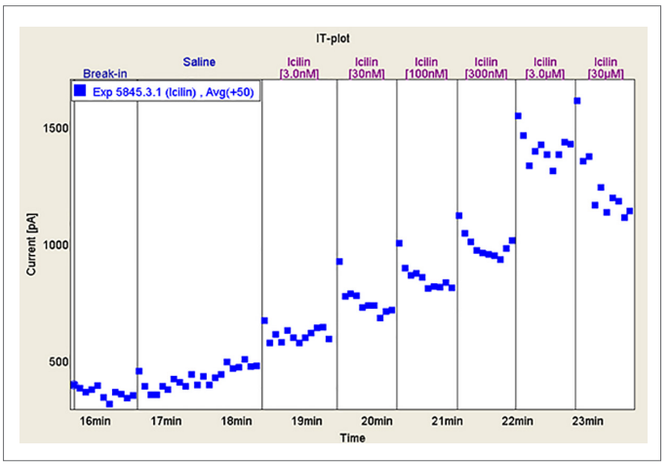


Fig. 10. IT-plot of the current elicited during the ramp (shown in Figure 9) at +50 mV in response to increasing concentrations of icilin.

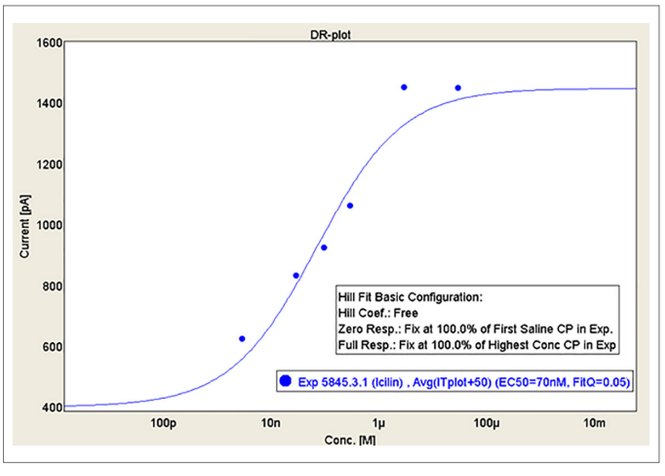


Fig. 11. Hill fit of the concentration-response relationship of icilin at V = +50 mV). EC_{50} was estimated to 70 nM.

Desensitization to the menthol signal was very evident when experiments were performed in the ligand-gated mode with a holding potential of -50 mV. In Figure 12 shows the current response elicited using repeated stimulation of the channel with (-)-menthol. Time between each stimulation was 2 min and up to ten washing steps were used to remove menthol again. Although the TRPM8 channel desensitized over time, such that the amplitude of the response was not reproducible.

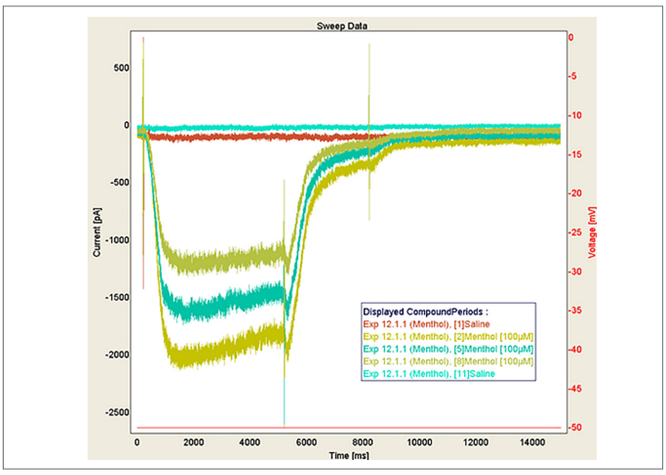


Fig. 12. Representative current traces of 3 consecutive (-)-menthol-evoked applications, recorded at a holding potential of -50 mV.

Success rates & Performance

Generally, the CHO-TRPM8 cell line performed very well on the QPatch, with high quality seals and long whole-cell lifetimes. However, different Ringer's solutions had a large impact on the success rate. Figure 13 gives an overview of the success rates obtained with three different Ringer pairs: IC0.0.0/EC0.0.0, EC TRPM8/IC TRPM8, and EC TRPM8 with K-based IC TRPM8.

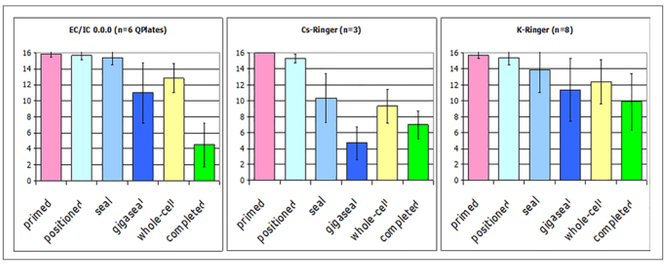
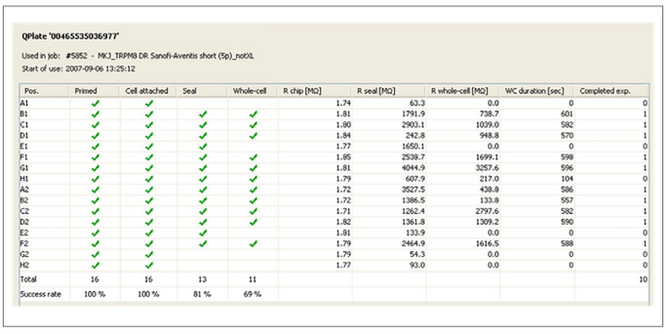


Fig. 13. Success rates ±SD of CHO-TRPM8 cells with three different Ringer pairs: IC0.0.0/EC0.0.0, TRPM8 EC/IC, and EC TRPM8 with K-based IC TRPM8. Each individual step in the protocol is represented, with the absolute number of sites on the y-axis (experiments were conducted on a QPatch 16): Number of primed sites on the QPlate, number of sites with cell positioning, number of sites with seals >100 MΩ, number of sites with real gigaseal (>1 GΩ), number of sites that have obtained whole-cell configuration, and number of completed experiments.

The CHO-TRPM8 cells performed best in the solution pair where cesium had been substituted for potassium. We experienced some problems when making the cesium-based Ringer, in that it precipitated with the chelating agent (EGTA or BAPTA). This is most likely the cause of the low sealing rates obtained in experiments with this Ringer.

Figure 14 shows an overview of the performance for one representative QPlate, taken from the QPatch Assay Software.



QPlate '0046553503697?'

Used in job: #5852 - MKJ_TRPM8 DR Sanofi-Aventis short (Sp)_notXL

Start of use: 2007-09-06 13:25:12

Pos.	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [sec]	Completed exp.
A1	✓	✓			1.74	63.3	0.0	0	0
B1	✓	✓	✓	✓	1.81	1791.9	738.7	601	1
C1	✓	✓	✓	✓	1.80	2903.1	1029.0	582	1
D1	✓	✓	✓	✓	1.84	242.8	948.8	570	1
E1	✓	✓	✓		1.77	1650.1	0.0	0	0
F1	✓	✓	✓	✓	1.85	2538.7	1699.1	598	1
G1	✓	✓	✓	✓	1.81	4044.9	3257.6	596	1
H1	✓	✓	✓	✓	1.79	607.9	217.0	104	0
A2	✓	✓	✓	✓	1.72	3527.5	438.8	586	1
B2	✓	✓	✓	✓	1.72	1386.5	133.8	557	1
C2	✓	✓	✓	✓	1.71	1262.4	2797.6	582	1
D2	✓	✓	✓	✓	1.82	1361.8	1309.2	590	1
E2	✓	✓	✓		1.81	133.9	0.0	0	0
F2	✓	✓	✓	✓	1.79	2464.9	1616.5	588	1
G2	✓	✓			1.79	54.3	0.0	0	0
H2	✓	✓			1.77	93.0	0.0	0	0
Total	16	16	13	11					10
Success rate	100 %	100 %	81 %	69 %					

Fig. 14. . Representative QPlate overview taken from the QPatch Assay Software showing number of primed sites, cell attachments, seals and wholecells, and the seal resistances measured at priming (R chip), gigaseal formation (R seal) and whole-cell (R whole-cell).

Results – HEK-hTRPM8

The HEK-hTRPM8 cell line was tested for comparison of the single-hole and multi-hole technology on QPatch. The multi-hole technology allows recordings of ten patched cells in parallel. Because the multi-hole QPlate is based on the same silicon chip technology of the single-hole QPlate, the data is of the same high quality.

Figure 15 and 16 show data from a representative dose-response experiment with the agonist -/-menthol and the antagonist capsazepine, respectively, in single-hole and multi-hole mode. Figure 17 summarizes the resulting EC/IC_{50}.

At very high agonist/menthol concentrations (>100 µM) the ion channel response is saturated for HEK-hTRPM8, and even decreases in most cases. In the CHO-TRPM8 cell line, the ion channel does not saturate until the menthol concentration reaches 500 µM. The reason for this difference is unknown.

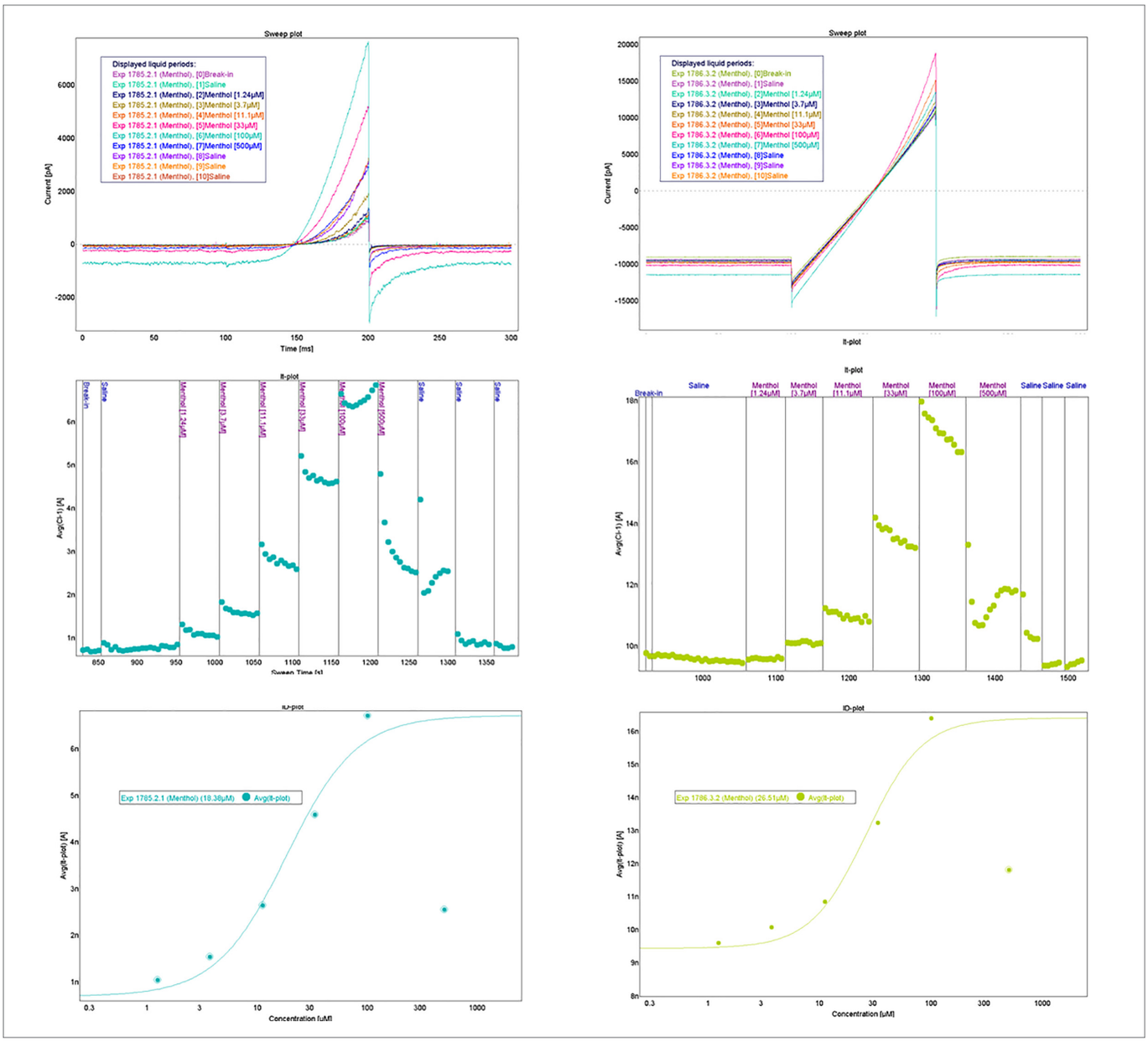


Fig. 15. Left: Single-hole data in a dose-response experiment with -/-menthol. Right: multi-hole data in a dose-response experiment with -/-menthol. Top: raw data sweeps elicited with a ramp from -80 mV to +60 mV, middle: IT plot of current at +60 mV, bottom: Hill fit.

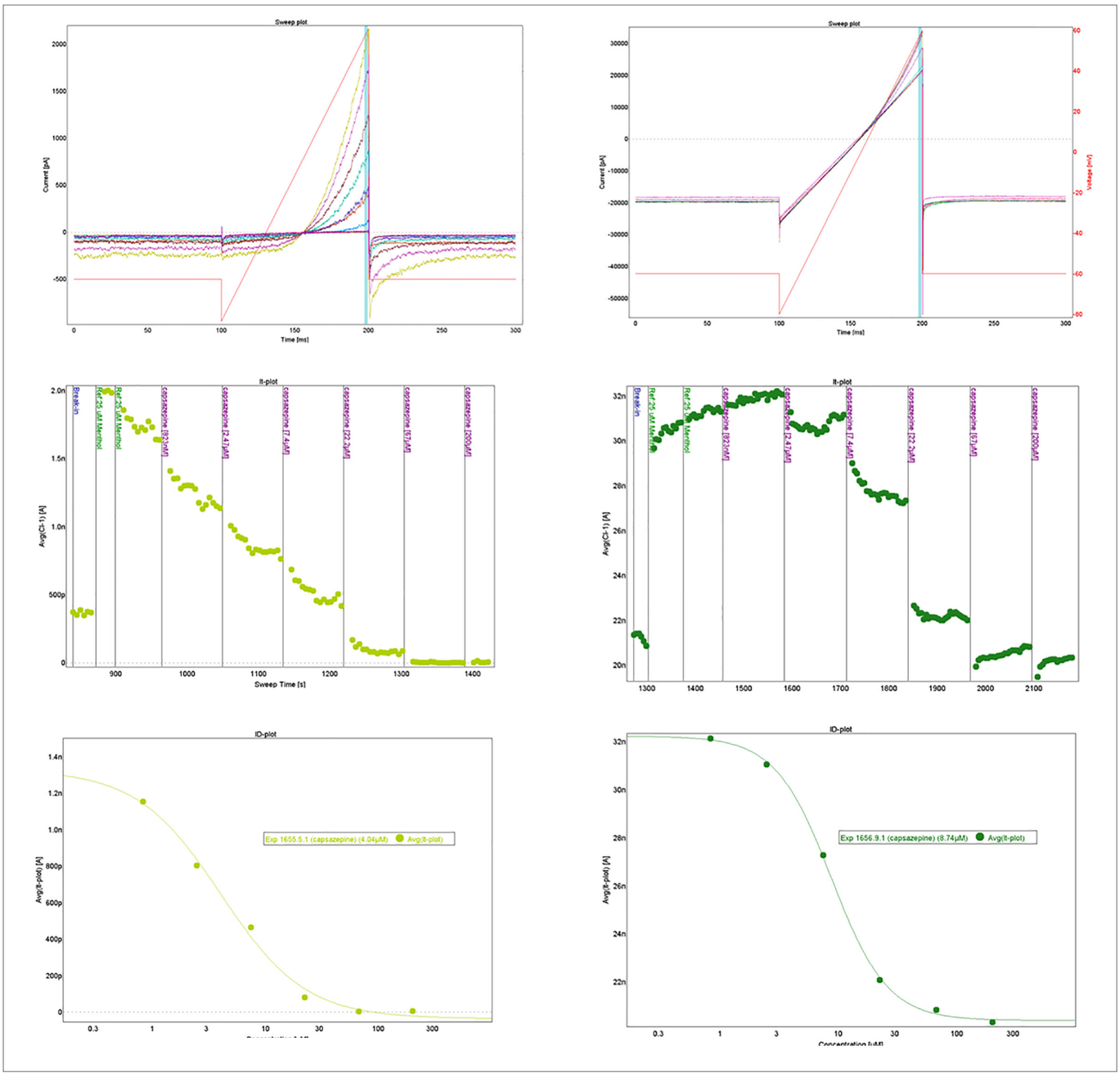


Fig. 16. Dose-response experiment with capsazepine tested in the presence of 25 µM -/-menthol. Left: Single-hole data in a dose-response experiment with capsazepine. Right: multi-hole data in a dose-response experiment with capsazepine. Top: raw data sweeps elicited with a ramp from -80 mV to +60 mV, middle: IT plot of current at +60 mV, bottom: Hill fit.

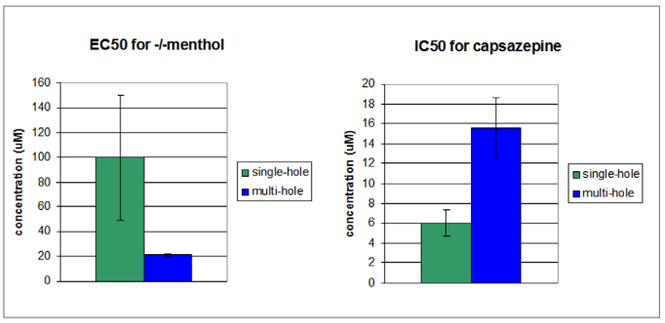


Fig. 17. EC_{50} ± SD values obtained with –(-)-menthol and capsazepine in single-hole and multi-hole mode (n=3-10).

Conclusion

CHO-TRPM8 and HEK-hTRPM8 cells perform well, and with high succes rates on QPatch. The QPatch can produce high-quality gigaseals and good recordings of the ion channel current. EC_{50} and IC_{50} estimations were successfully obtained for menthol, icilin and capsazepine, and were in good agreement with values reported in the literature. We further demonstrate that TRPM8 can be measured using multi-hole QPlates using QPatch multi-hole technology.

Methods

Electrophysiology

Currents were elicited using two different voltage protocols (the voltage protocol can be seen in all raw data plots in the following figures). To monitor compound effects over time, a 100 ms ramp from -80 mV to +80 mV was repetitively applied. A current voltage (IV) relationship was recorded using, a sequence of 400 ms long voltage pulses from -120 mV to +200 mV in 20 mV increments from V_{hold} = -100 mV.

Cell culture

Cells were grown according to Sophion`s SOP for CHO cells. Cell growth medium was Hams F12 supplemented with 10 % Fetal bovine serum.

References:

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