

Application Report

HEK293-Ca_v2.2 for QPatch

Here we present results from a validation study for a pharmaceutical company on currents from the N-type voltage gated calcium channels (Ca_v2.2) studied on QPatch

Summary

We tested the HEK293-Ca_v2.2 cell line on QPatch. High seals and gigaseal resistances are readily obtained with these cells, and the establishment of the whole cell configuration seems to be rather straightforward. Not all measurements were usable due to low current level and leaky whole cells. Both Compound A and flunarizine were found to block the Ca_v2.2 current and behave as expected. Based on these results, the pharmaceutical company purchased a QPatch.

Introduction

It is common that potential customers ask Sophion Bioscience to use QPatch to test some of their compounds on specific ion channels before buying a system. In this application report, we present some of the results obtained from a validation study done for a pharmaceutical company. Currents from the N-type voltage gated calcium channels (Ca_v2.2) were studied using QPatch. A blocker of the Ca_v2.2 current, compound A, was applied in increasing concentrations to study the dose-response effects and estimate IC₅₀ values. Furthermore, flunarizine which is known to block T-type Ca²⁺/Na⁺ channels and have low potency on N-type Ca channels, was tested.

Results

Compound A was diluted in extracellular Ringer solution and tested in 4 concentrations (0.74, 2.22, 6.67, 20 μM). The resulting current can be seen in Figure 1. The mean IC₅₀ value for peak 1 (first depolarization, Figure 2) was determined to be 5.7 μM ± 3.5 μM (n=7). The mean IC₅₀ value for peak 2 (the last depolarization, Figure 3) was determined to be 4.6 μM ± 2.4 μM (n=6). The expected IC₅₀ values were in the range of 1-10 μM, and the results were therefore acceptable.

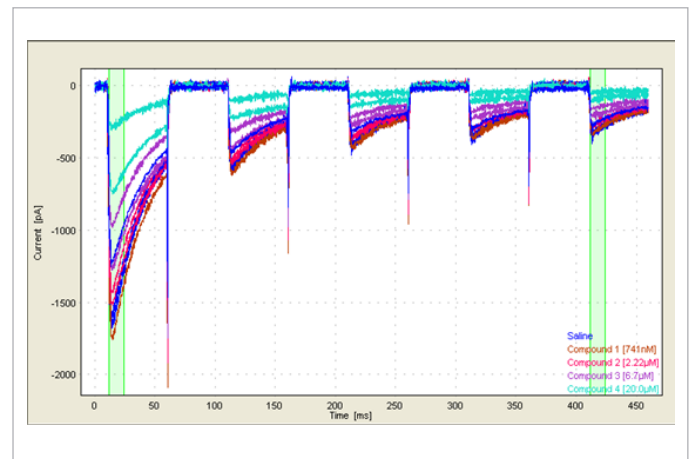


Fig. 1: Ca_v2.2 current elicited using the voltage protocol shown in Figure 5.

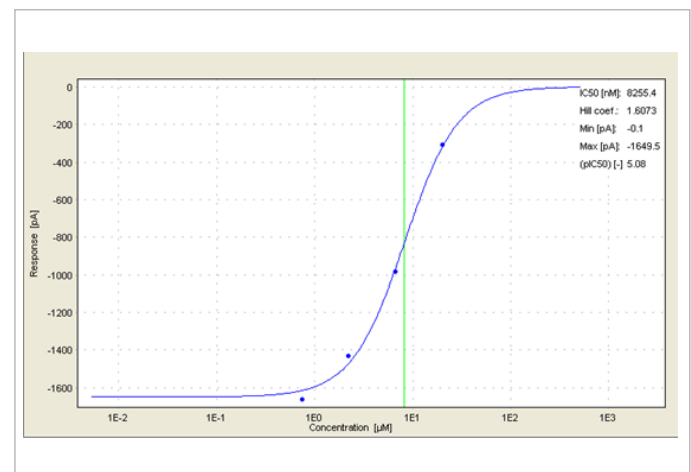


Fig. 2: Hill fit of peak current from first depolarization.

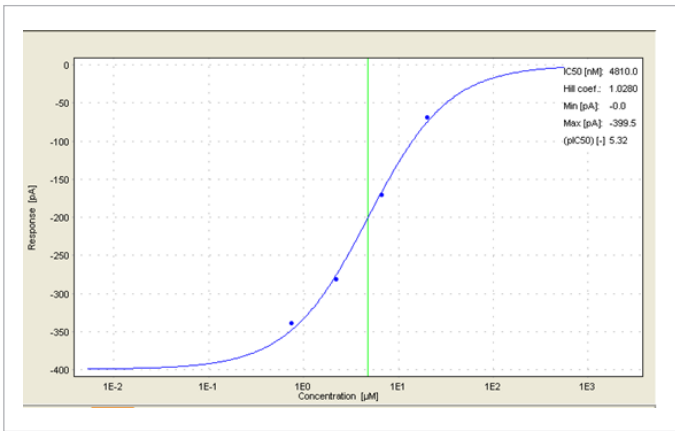
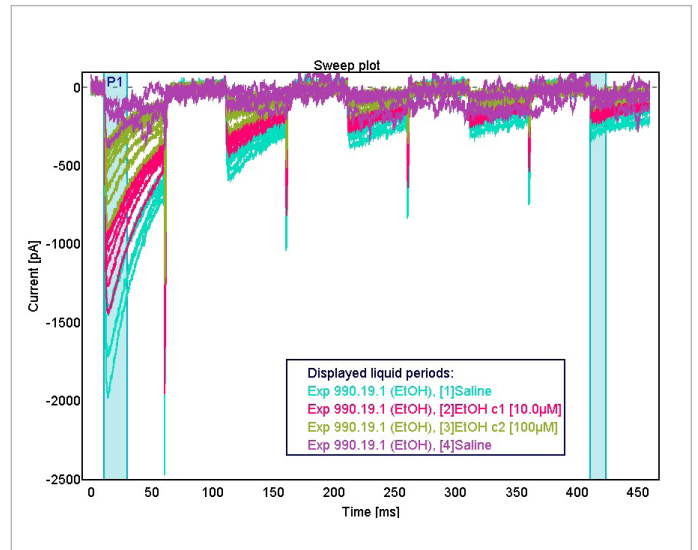


Fig. 3: Hill fit of peak current from last depolarization.



HEK293 Cav2.2 current blocked by flunarizine

Flunarizine is a sticky compound that is difficult to dissolve. In these experiments flunarizine was dissolved in EtOH. The stock solution was diluted in extracellular ringer, and the concentrations 10 μM and 100 μM were tested. In this example, 100 μM flunarizine blocked 80.8% of the baseline maximal peak current (blue dots in Figure 5). The IC_{50} value of this experiment was determined to be 13.5 μM . Flunarizine tends to precipitate, which can be avoided using DMSO as vehicle and a phosphate free external solution.

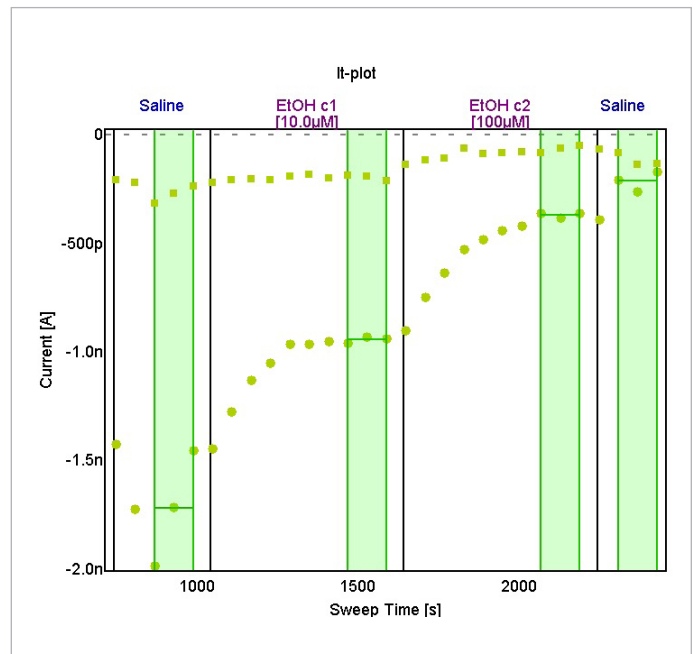


Fig. 4: HEK $\text{Ca}_v2.2$ current blocked by flunarizine at 10, 100 μM dissolved in EtOH



Seal quality and stability

In general, the HEK293-Ca_v2.2 cells seal easily and many whole cells are obtained per QPlate (Table 1).

Table 1: QPlate overview showing seal quality and whole-cell lifetime.

QPlate '00465535024869'

Used in job: #646 - RS_Cav2.2-SHORT-with RS_4

Start of use: 2006-11-14 13:26:20

Pos.	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [sec]	Completed exp.
A1	✓	✓	✓		1.72	301.2	0.0	0	0
B1	✓	✓	✓		1.65	70.1	0.0	0	0
B2	✓	✓	✓	✓	1.63	2771.1	1223.8	832	8
D1	✓	✓	✓	✓	1.77	2326.2	3402.5	877	1
E1	✓	✓	✓	✓	1.67	8722.8	4129.0	868	1
F1	✓	✓	✓	✓	1.63	5848.1	1401.3	895	1
G1	✓	✓	✓	✓	1.63	1102.8	821.2	847	0
H1	✓	✓	✓	✓	1.64	148.6	933.9	885	1
A2	✓	✓	✓		1.66	1861.4	1061.6	894	1
B2	✓	✓	✓		1.65	2720.9	0.0	0	0
C2	✓	✓	✓		1.70	51.4	0.0	0	0
D2	✓	✓	✓		1.73	50.5	0.0	0	0
E2	✓	✓	✓	✓	1.64	4902.0	6599.1	894	1
F2	✓	✓	✓		1.64	49.6	0.0	0	0
G2	✓	✓	✓	✓	1.85	3227.8	1589.9	906	1
H2	✓	✓	✓	✓	1.65	184.5	390.6	450	0
Total	16	16	12	10					8

Success rate: 100 % 100 % 75 % 63 %

Methods

HEK293 cells stably expressing the Ca_v2.2 current were grown in DMEM with 10% FBS.

Compound A was received from the pharmaceutical company. Flunarizine dihydrochloride (1-[Bis(4-fluorophenyl)methyl]-4-(3-phenyl-2-propenyl)piperazine dihydrochloride) was obtained from Sigma (F8257).