Application Report:

HEK-$Ca_V3.2$

Recordings on QPatch

AR_PUBLIC14469-3

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

HEK-293-$Ca_V3.2$

Introduction

The QPatch16 automated patch clamp system was used to establish and record currents from HEK293 cells stably expressing $Ca_V3.2$ channels. Whole cell currents were measured upon application of a voltage-step protocol to obtain I-V curves.

Materials & Methods

Ringer's Solutions

Internal Ringer (in mM): CsF (140), EGTA (1), HEPES (10), NaCl (10).

External Ringer (in mM): TEACl (157), $MgCl_2$ (0.5), $CaCl_2$ (5), HEPES (10).

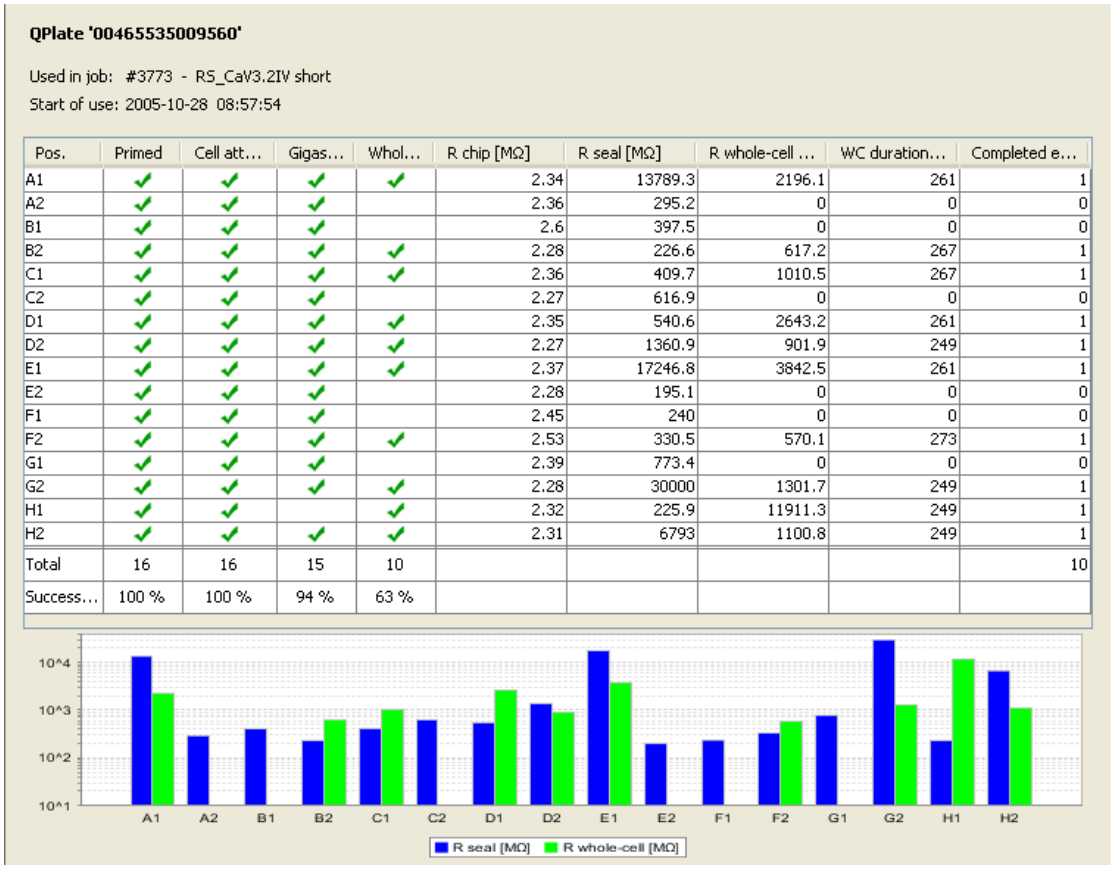
Cells

The cells used in these experiments were HEK293 stably expressing $Ca_V3.2$ channels. The cells were grown in standard media for HEK293 cells. Prior to use, the cells were maintained in the QPatch cell storage facility in suspension. Shortly before the experiment the cells were automatically transferred to the QPatch mini centrifuge, spun down and washed once, before being resuspended in the external Ringer's solution and transferred to the pipetting wells in the QPlate.

Results

QPlate Summary

Using QPatch16 an overview of the results from each QPlate can be extracted. This overview show e.g. the number of primed sites, number of cells attached to the measurement sites, number of giga seals, number of whole cells, and number of completed experiments. Figure 1 shows a typical overview obtained after an experiment with HEK293 cells expressing $Ca_V3.2$ channels.



QPlate '00465535009560'

Used in job: #3773 - RS_CaV3.2IV short
Start of use: 2005-10-28 08:57:54

Pos.	Primed	Cell att...	Gigas...	Whol...	R chip [MΩ]	R seal [MΩ]	R whole-cell ...	WC duration...	Completed e...
A1	✓	✓	✓	✓	2.34	13789.3	2196.1	261	1
A2	✓	✓	✓		2.36	295.2	0	0	0
B1	✓	✓	✓		2.6	397.5	0	0	0
B2	✓	✓	✓	✓	2.28	226.6	617.2	267	1
C1	✓	✓	✓	✓	2.36	409.7	1010.5	267	1
C2	✓	✓	✓		2.27	616.9	0	0	0
D1	✓	✓	✓	✓	2.35	540.6	2643.2	261	1
D2	✓	✓	✓	✓	2.27	1360.9	901.9	249	1
E1	✓	✓	✓	✓	2.37	17246.8	3842.5	261	1
E2	✓	✓	✓		2.28	195.1	0	0	0
F1	✓	✓	✓		2.45	240	0	0	0
F2	✓	✓	✓	✓	2.53	330.5	570.1	273	1
G1	✓	✓	✓		2.39	773.4	0	0	0
G2	✓	✓	✓	✓	2.28	30000	1301.7	249	1
H1	✓	✓		✓	2.32	225.9	11911.3	249	1
H2	✓	✓	✓	✓	2.31	6793	1100.8	249	1
Total	16	16	15	10					10
Success...	100 %	100 %	94 %	63 %					

Figure 1. QPlate summary.

Current Sweeps

After gigaseal and whole cell requirements are accomplished according to the specifications dictated by the user-defined whole cell protocol (not shown), currents are generated according to the user-defined voltage protocol (see Figure 2).

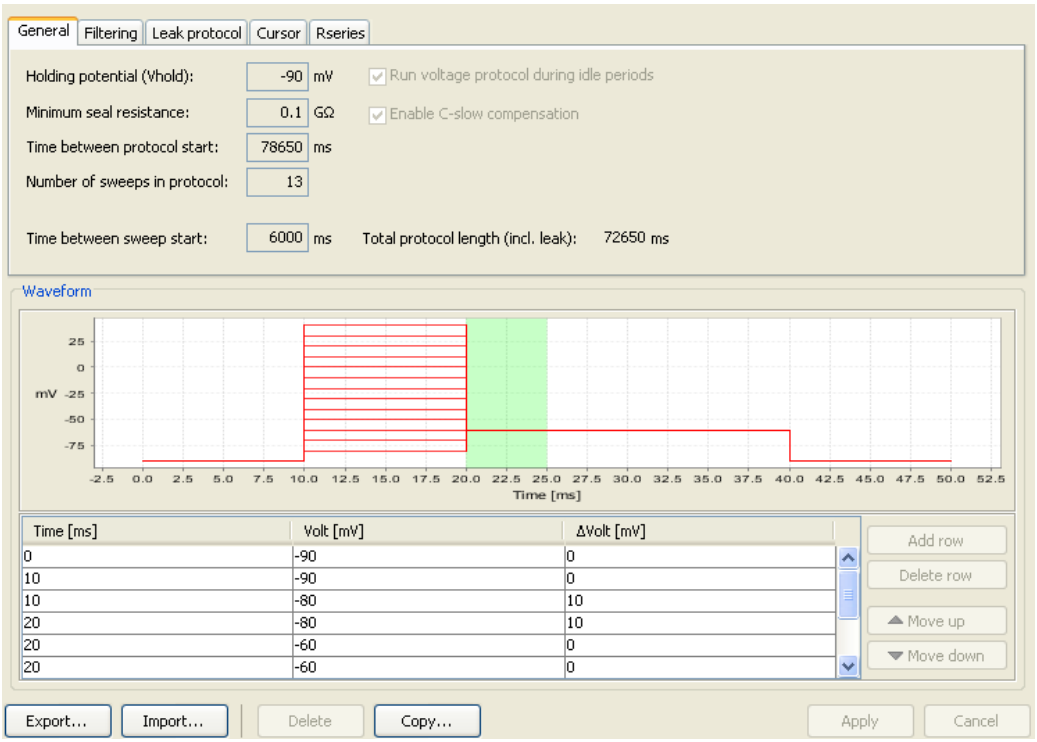


Figure 2. Voltage protocol.

In these experiments we stimulated the HEK293-CaV3.2 cells with potentials ranging from -80 to +40 mV (10 ms duration) from a holding potential at -90 mV (see Figure 2 for specifications). Typical whole cell current sweeps are shown in Figure 3.

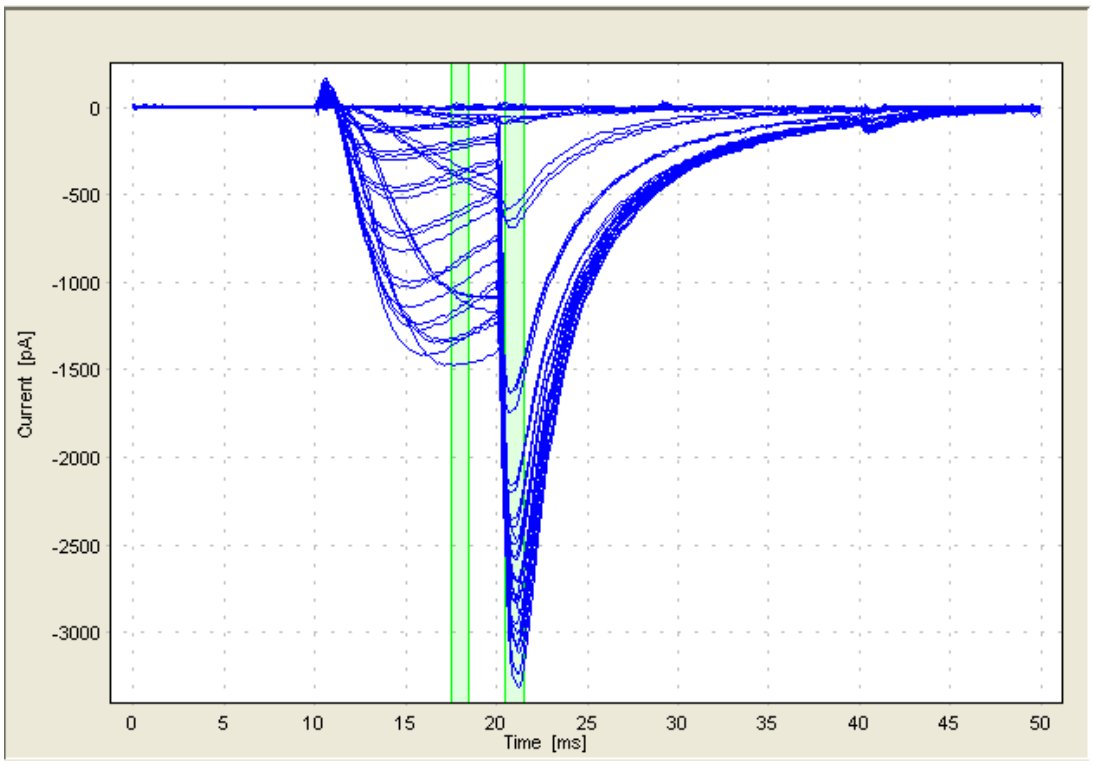


Figure 3. Ca$_V$3.2 whole-cell current.

I-V curve

In this experiment the voltage protocol was applied three times, as specified in the user-defined application protocol (not shown). No compounds were applied in this experiment, thus the I-V curve for the control current is shown in Figure 4. As can be seen from the figure the current-voltage relationship is characteristically bimodal with the current activated at potentials > -60 mV. The maximal current was observed at – 20 mV which is consistent with the literature describing the CaV3.2 whole-cell currents obtained from HEK293 cells (Perchenet et al. 2000).

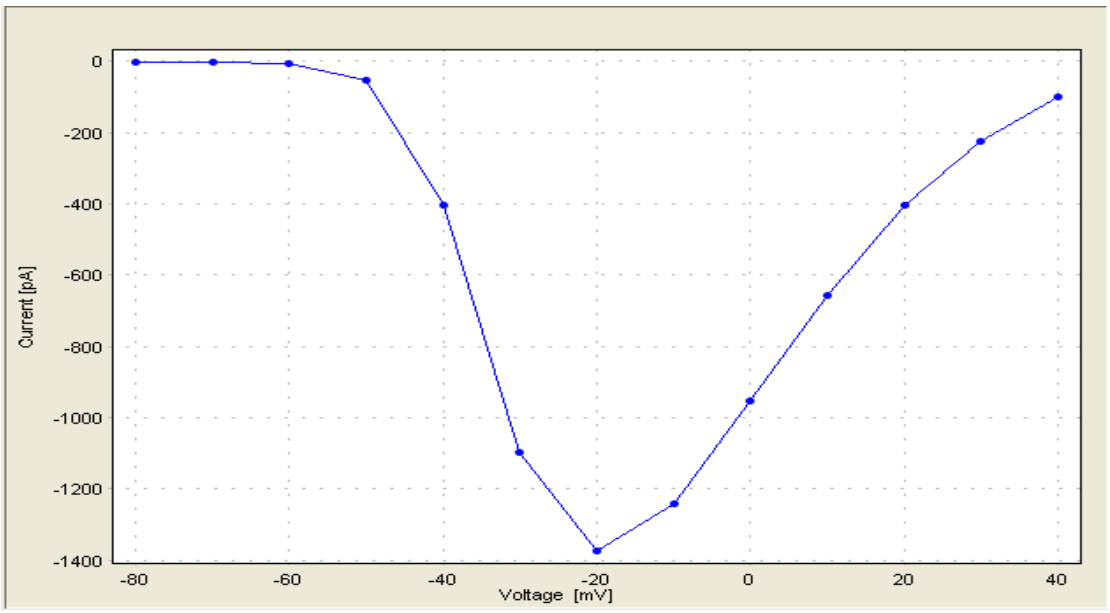


Figure 4. The average I-V curve from three voltage-step stimulations of the cell. For the current amplitude analysis the left cursor interval depicted in Figure 3 (17-18 ms) was used.

Tail Current

The tail current was plotted as a function of the step potential (see Figure 5) and the interpolated V0.5 was approximately -30 mV which is consistent with the value reported from the literature (Perchenet et al. 2000).

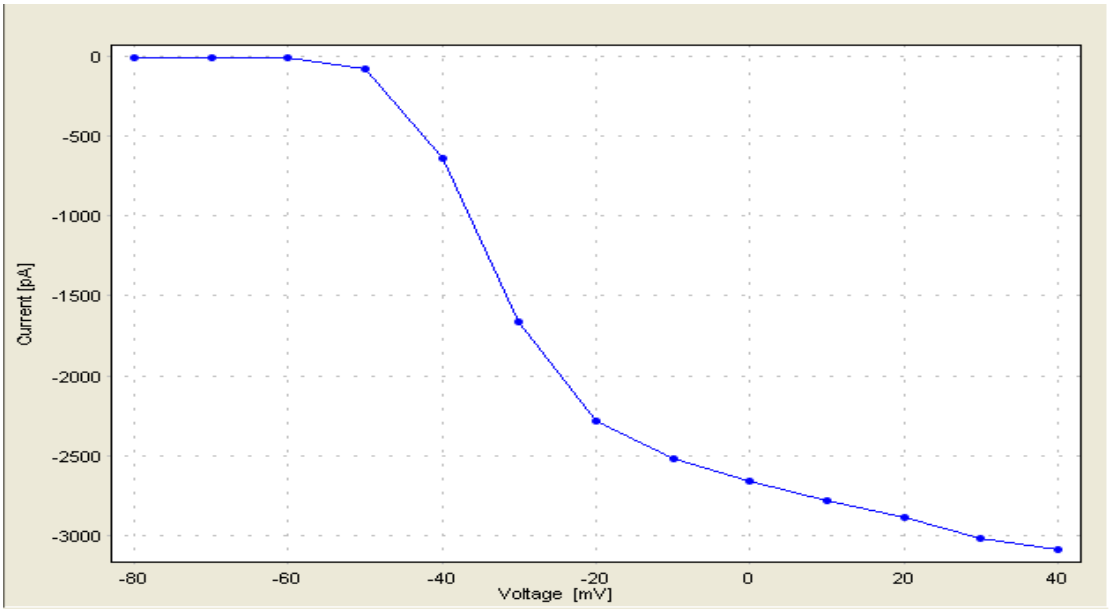


Figure 5. Tail current as a function of the step potential. The current amplitude was measured using the right cursor interval in Figure 3. The average current from the three voltage-step stimulations is plotted.

References

Perchenet L, Benardeau A, Ertel EA (2000) Pharmacological properties of Ca(V)3.2, a low voltage-activated Ca2+ channel cloned from human heart. Naunyn Schmiedebergs Arch Pharmacol. 361(6):590-9

Conclusion

With the QPatch technology we have obtained high seal resistances and stable whole cells on HEK293 cells expressing CaV3.2 channels. The obtained current characteristics were highly comparable to the characteristics reported in the existing literature for CaV3.2. Therefore, we conclude that the HEK293-CaV3.2 cell line is highly suitable for screening as well as research purposes performed on QPatch.