Application Note

Channel: $Ca_V2.2$
Cells: HEK293
Tools: Port-a-Patch®
External Perfusion

Characterization of $Ca_V2.2$ (HEK293) on Nanion‘s Port-a-Patch®

The electrophysiology team at Nanion Technologies GmbH, Munich.
Cells were supplied by Millipore, USA

Summary

The voltage gated N-type calcium channel ($Ca_V2.2$) is encoded by the gene CACNA1B. $Ca_V2.2$ is a high voltage activated calcium channel.
$Ca_V2.2$ is found mainly in the brain, where it mediates neurotransmitter release at the synapse. The strong depolarization of neuronal action potentials causes the opening of the channel. Calcium can then enter the cell and initiates the fusion of the neurotransmitter vesicles with the membrane.
$Ca_V2.2$ is inhibited by ω-conotoxin, a neurotoxin of the fish hunting snail, with high specificity. CaV2.2 has been implicated in the transmission of pain. Pharmacological block of $Ca_V2.2$ by compounds based on ω-conotoxin has been shown to be effective against strong chronical pain.
The biophysical and pharmacological properties of the cells are presented in this Application Note.

Results

Figure 1 shows current responses of an individual cell to a current-voltage relationship step protocol. Potentials were stepped from the holding potential (-80 mV) for 50 ms to the test potential before stepping back to holding. Test potentials were varied between -20 mV and 90 mV in 10 mV increments.

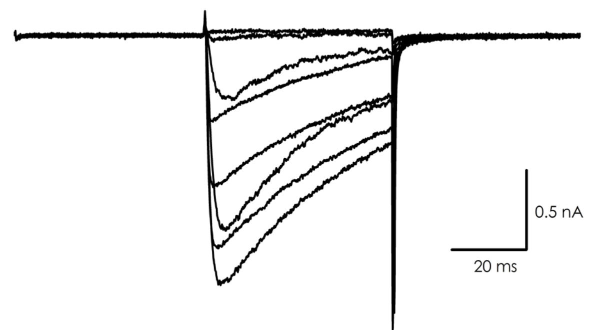

Figure 1:
Representative current responses of an individual cell expressing $Ca_V2.2$ to a Ca_V IV voltage protocol (for details see text).

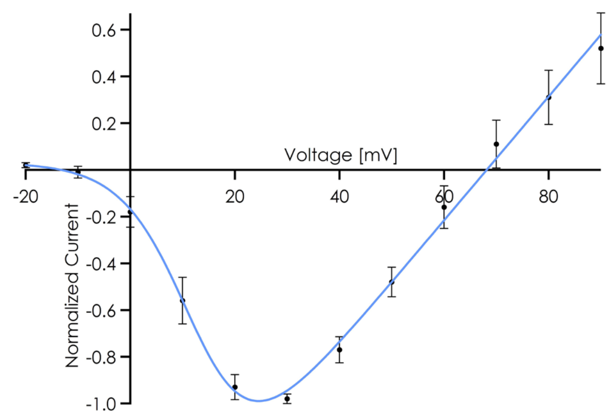

Figure 2:
Average current-voltage relationship (n = 10). The error bars reflect the standard error of the mean (S.E.M.).

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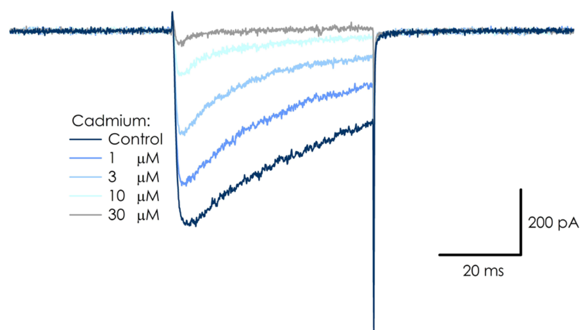

Figure 3:
Shown are raw current traces from an individual cell expressing $Ca_V2.2$ under control conditions (black) and at increasing concentrations of cadmium as indicated.

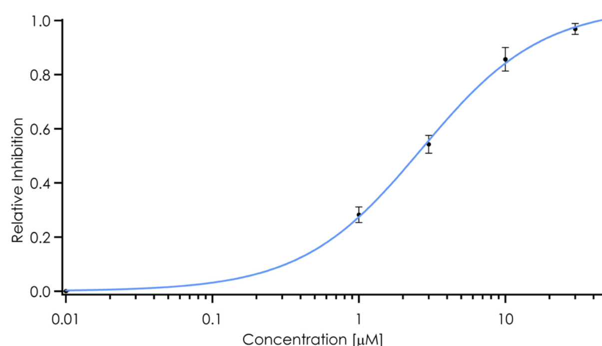

Figure 4:
Shown are raw current responses in the presence of increasing cadmium concentrations as indicated.

Figure 2 shows the average current-voltage relationship for ten cells. The average mean current at 30 mV of all recorded cells was -744 ± 140 pA (n = 10).
The inhibition of $Ca_V2.2$ by cadmium was studied using the External Persusion System. Figure 3 shows the current of an individual cell at increasing concentrations of cadmium (as indicated). The average dose-response curve is shown In Figure 4. From this the IC_{50} was calculated to be 2.7 ± 0.4 µM (n = 5).
The inhibition by cadmium was fully reversible, as can be seen in Figure 5. For the cell shown, cadmium was washed out and applied at its highest concentration another three times after collection of a full dose-response curve. Control current levels were recovered each time cadmium was washed out.

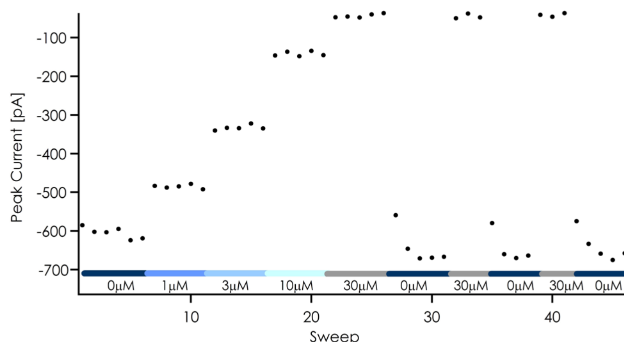

Figure 5:
Block by cadmium is fully reversible. Shown is the timecourse of the peak Cav 2.2 current with the application of cadmium as indicated.

Methods

Cell Culture

HEK293 cells stably expressing Cav2.2 were supplied by Millipore. Cells were cultured and harvested according to Nanion's standard cell culture protocol.

Solutions

Standard patch clamp solutions were modified for the Ca^{2+} channel recordings. The external solution contained 20 mM Ba^{2+} as the charge carrier. The predominant ion in the internal solution was Cs^{+} .It also contained Mg-ATP, GTP, and BAPTA to minimize current run down.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Port-a-Patch®. Currents were elicited using a voltage step from a holding potential of -80 mV for 50 ms to different test potentials and back to holding. Pulses were elicited every 10 seconds.

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