Application Note

Channel:	$Ca_V2.2$
Cells:	HEK293
Tools:	Patchliner®

Characterization of $Ca_V2.2$ (HEK293) on Nanion's Patchliner®

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) to the test potential for 50 ms before stepping back to holding. Test potentials were varied between -20 mV and 90 mV in 10 mV increments.
Figure 2 shows the average current-voltage relationship of ten cells. The average peak current at 30 mV of all recorded cells was -698 ± 115 pA (n = 6).

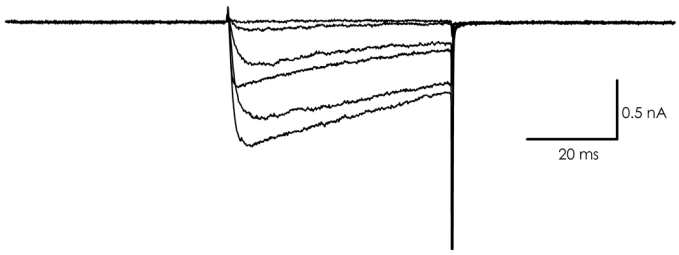

Figure 1:
Representative current responses of an individual cell expressing $Ca_V2.2$ to a Ca_V IV voltage protocol (for details see text).

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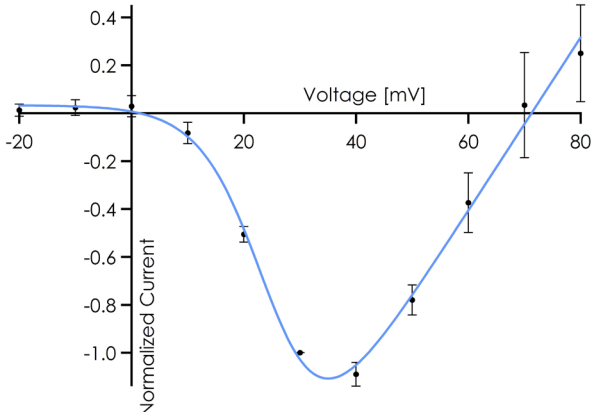

Figure 2:
Average current- voltage relationship (n = 10). The error bars reflect the S.E.M..

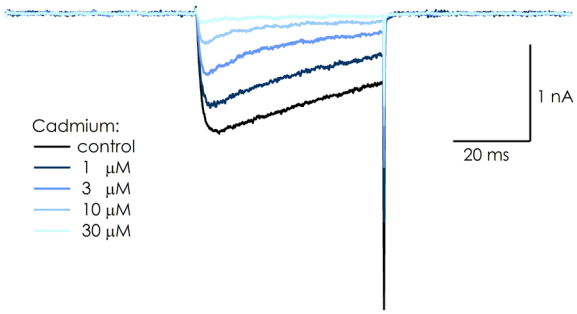

Figure 3:
Shown are raw current traces from an individual cell expressing $Ca_V2.2$ under control conditions (black) and at increasing concentrations of cadmium (as indicated).

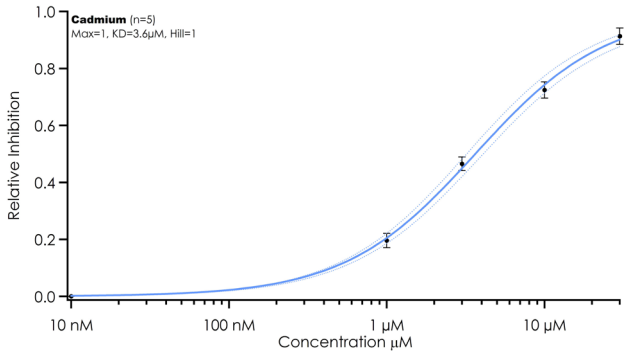

Figure 4:
Average cadmium dose-response curve. Error bars represent the S.E.M.

Figure 3 shows the current response of an individual cell in the presence of increasing cadmium concentrations. The average dose-response curve for block of $Ca_V2.2$ by cadmium is shown in Figure 4. The IC_{50} was calculated from its Hill fit to be 3.6 ± 0.4 µM (n = 5).

Given the importance of $Ca_V2.2$ in pain pathways and in the treatment of chronic pain, a planar patch clamp device with increased throughput is an invaluable tool to study this target. The Patchliner's low compound consumption is just one of its critical features that makes it the perfect tool for the $Ca_V2.2$ target where low volumne toxins are likely drug candidates.

Methods

Cells

HEK293 cells stably expressing $Ca_V2.2$ were kindly supplied by Millipore.

Cell culture

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 measurements. 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 Patchliner®. 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.

Nanion Technologies GmbH
Erzgiessereistr. 4
80335 Munich, Germany

phone +49 89 218997972
fax +49 89 218997960
www.nanion.de • info@nanion.de