Application Note

Channel: $Ca_V3.2$
Cells: HEK293
Tools: Patchliner®

Characterization of $Ca_V3.2$ (HEK293) on Nanion's Patchliner®

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

Summary

The gene CACNA1H encodes the a_{1H} subunit of the voltage-gated calcium channel $Ca_V3.2$. It belongs to the low voltage-activated T-type calcium channels. $Ca_V3.2$ displays the typical characteristics of the T-type channels: activation at low depolarization of the membrane and transient kinetics.
T-type Ca^{2+} channels are involved in diverse, mainly rhythmic processes like e.g. pacemaking and generation of thalamocortical rhythms in sleep or epilepsy. $Ca_V3.2$ is expressed in a wide variety of cells. Amongst others it has been found in kidney, smooth muscle, brain, adrenal and cardiac cells. It seems to be involved in contraction of smooth muscle and the secretion of the adrenal hormones aldosterone and cortisol. Pharmacological block of T-type channels may lead to new drugs for the treatment of hypertension and epilepsy.
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 -50 mV and 40 mV in 10 mV increments.

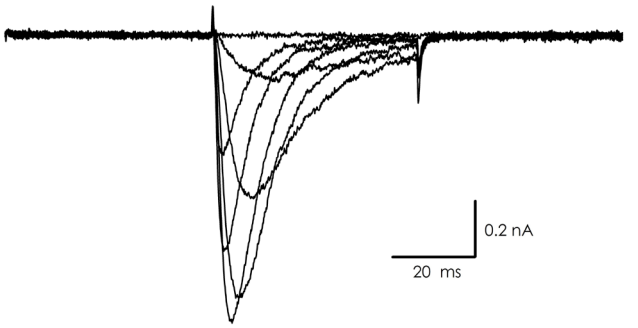

Figure 1:
Representative current responses of an individual cell expressing $Ca_V2.2$ to a Ca_V IV voltage protocol (for details see text).

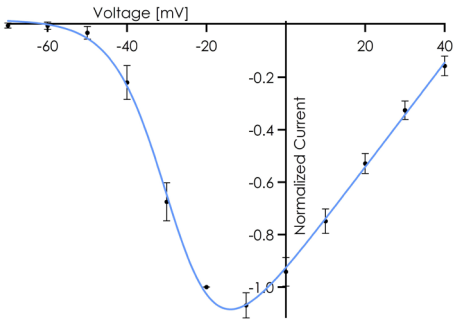

Figure 2:
Average current- voltage relationship (n = 12). The error bars reflect the S.E.M.

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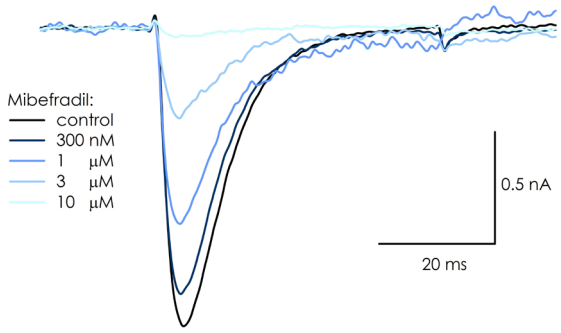

Figure 3:
Shown are raw current traces from an individual cell expressing Ca_V 3.2 under control conditions (black) and at increasing concentrations of mibefradil as indicated.

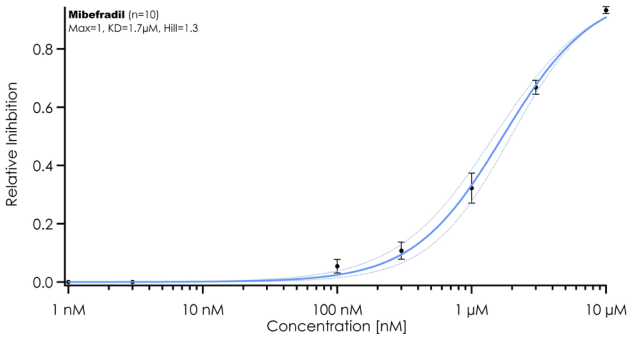

Figure 4:
Shown is the average dose-response curve for mibefadril. Error bars depict the S.E.M..

Figure 2 shows the average current-voltage relationship of 12 cells. The average mean current at -20 mV of all recorded cells was -785 ± 110 pA (n = 12).
In pharmacological experiments the effect of mibefadril was determined. Figure 3 shows raw current traces of an individual cell in the absence and presence of increasing concentrations of the compound. The average dose-response curve is shown in Figure 4.
The voltage dependence of channel inactivation was also examined (Fig 5). Current responses of a double pulse protocol with varying test potentials between the pulses (5 s) was used to determine the half inactivating potential. Peak current responses to the second pulse are expressed relative to the response to the first pulse. This normalized current and plotted against the voltage applied between the pulses. The activation curve (Fig 5)

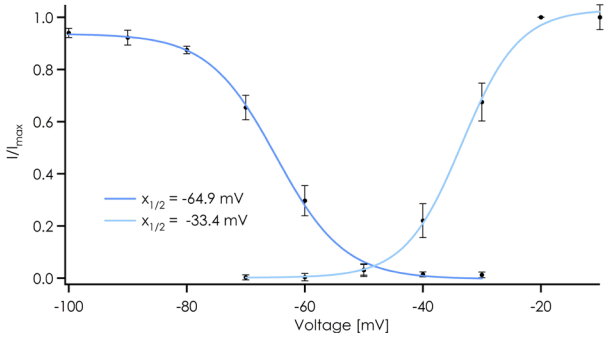

Figure 5:
Average activation and inactivation curves for Ca_V3.2 as determined from a single and double pulse protocol, respectively.

was constructed from a regular IV step protocol (Fig 1). Both curves in Figure 5 were fitted with the Boltzmann equation and revealed a half-inactivating potential of -65 mV and a half-activating potential of -33 mV.

Methods

Cells/Cell Culture

HEK293 cells stably expressing Ca_V3.2 were kindly supplied by Millipore. Cells were cultured and harvested according to Nanion's standard cell culture protocol.

Solutions

Standard patch clamp solutions were used modified for 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 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.

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