Application Note

Ion Channels:
K_V7.2/7.3

Cells:
HEK293

Tools:
Patchliner

Cross-site study of neuronal K_V7.2/7.3 heteromers on the Patchliner

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Summary

The K_V7 channels are a family of voltage-gated potassium ion channels with five members (K_V7.1 - 7.5) encoded by the KCNQ1-5 genes[1]. The channels exist as tetramers, with each subunit containing six transmembrane domains with cytoplasmic N- and C-termini. The long intracellular terminus is essential for tetramermization as well as interaction with critical regulators such as PIP_2, calmodulin, protein kinase C and ankyrin G[2]. K_V7-mediated currents are voltage activated, slowly activating and non-inactivating and are involved in repolarization of the cell membrane potential, thereby controlling cell excitability[3]. K_V7.1 channels are primarily expressed in cardiac cells whereas K_V7.2, K_V7.3 and K_V7.5 are widely distributed in neuronal and primary sensory cells[2]. K_V7.2/7.3 heteromeric channels primarily underlie the neuronal M-current (I_{KM})which plays a crucial role in repolarizing neuronal membrane potential after a depolarizing input which limits repetitive firing and is, therefore, a key mechanism in spike frequency adaptation[4].

Mutations in KCNQ2 and KCNQ3 have been shown to cause benign familial neonatal convulsions (BFNC), a rare inherited form of epilepsy, and neonatal epileptic encephalopathy (NEE)[2,4]. Mutations in the gene can affect voltage dependence of the resulting channel, alter the interaction with PIP_2, affect the subcellular localization at key neuronal sites or cause loss of channel function[2]. Activation of K_V7.2/7.3 is, therefore, a potential strategy to treat epilepsy with retigabine (ezogabine) approved as an adjunct therapy in partial-onset seizures in 2011, but was later withdrawn from the market in 2017 due to adverse side effects. In addition to epilepsy, K_V7.2/7.3 has also been proposed as a potential target to treat pain, neuropsychiatric disorders such as anxiety, attention deficit–hyperactivity (ADHD), mania, bipolar disorder and schizophrenia

Results

K_V7.2/7.3 stably expressed in HEK293 cells were used on the Patchliner at two different sites, Nanion HQ and ApconiX. Current responses to increasing voltage steps were used to construct current-voltage plots. The curves were fit with a Boltzmann equation and the IV plot for an average of 18 cells recorded at ApconiX is shown in Figure 1A with traces from an example cell shown in the inset. The average V_{half} calculated from 18 individual plots was -19.9 ± 2.0 mV in excellent agreement with the literature[6]. At a second site (Nanion HQ), the average V_{half} calculated from 16 individual plots was -22.2 ± 1.8 mV showing excellent consistency across the two sites. The recordings were also highly stable with little change in peak amplitude over a 15 minute measurement period. Figure 1B

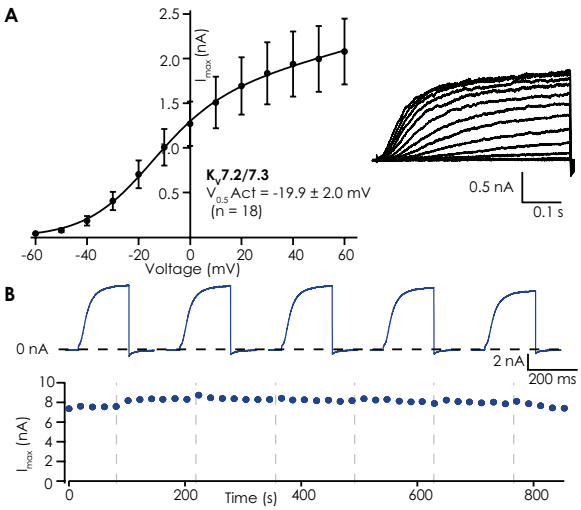

Figure 1: A *Current-voltage plot for K_V7.2/7.3 with representative traces.* **B** *Stability of K_V7.2/7.3 recordings. Currents could be recorded for over 15 minutes with little change in amplitude over time. Shown are raw traces from an exemplar cell and the corresponding time course in external solution.*

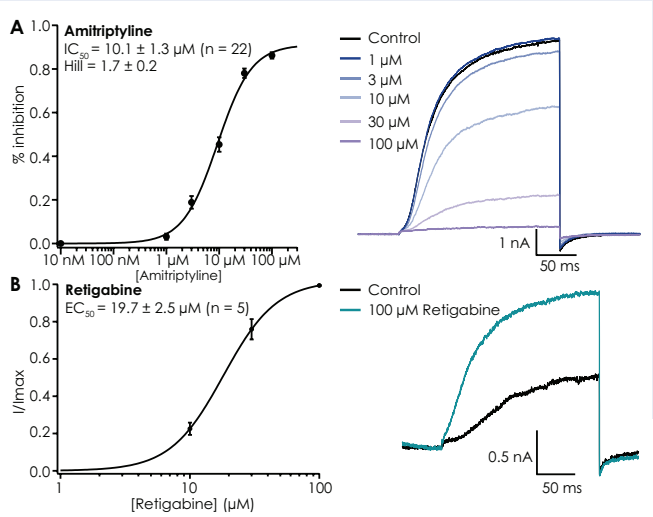

Figure 2: A *Amitriptyline inhibited K_V7.2/7.3 in a concentration-dependent manner. The CRC for an average of 22 cells is shown and traces from an example cell.* **B** *Retigabine enhanced K_V7.2/7.3-mediated responses. The CRC for an average of 5 cells is shown and example traces from one cell +/- retigabine.*

shows the timecourse and corresponding raw traces from an example cell.

Amitriptyline is a tricyclic antidepressant with analgesic and sedative properties, although it can be associated with severe side effects such as seizures[7]. It blocks both K_V1.1 and K_V7.2/7.3 channels which could underlie the neuroexcitatory side effects of the drug[7]. Amitriptyline blocked K_V7.2/7.3-mediated currents with an IC_{50} at one site (Nanion HQ; Figure 2A) of 10.1 ± 1.3 µM (n = 22) and at the second site (ApconiX) of 6.9 ± 1.2 µM (n = 8) in excellent agreement with each other (P>0.05, unpaired Student's t test) and with the literature[7].

Retigabine is an anticonvulsant that enhances K_V7.2/7.3-mediated responses by shifting the voltage-dependence of activation to more hyperpolarized potentials[8,9,10]. In our experiments, retigabine enhanced K_V7.2/7.3-mediated responses in a concentration-dependent manner with an EC_{50} = 19.7 ± 2.5 µM (n = 5). Figure 2B shows the concentration response curve (CRC) for an average of 5 cells and the traces for an example cell in the presence and absence of 100 µM retigabine.

In summary, K_V7.2/7.3 expressed in HEK cells was recorded on the Patchliner at two different sites with very comparable results for V_{half} and pharmacology. The Patchliner is an excellent tool for studying K_V7.2/7.3 and identifying novel anticonvulsants and analgesic compounds which enhance K_V7.2/7.3 activity.

References

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Methods

Cells

HEK293 cells stably expressing K_V7.2/7.3 were used.

Electrophysiology measurements

Cells were cultured and harvested according to Nanion's standard protocols. Cells were resuspended in external recording solution and stored in the CellHotel of the Patchliner before being dispensed into each well of the NPC-16 chip. Internal and external solution compositions are available upon request. For IV plots, voltage was increased in 10 mV steps from -60 mV to 60 mV from a holding potential of -80 mV. For amitriptyline, a single step to 60 mV from a holding potential of -80 mV was used and for retigabine, a single step to 0 mV was used. Data was analyzed in Igor or Prism. IV plots were fit with a Boltzmann equation and CRC plots with a Hill equation. Data is given as mean ± S.E.M.

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