Application Note

Ion Channels:
$Na_V1.7$, nAChR $\alpha1\beta1\gamma\delta$

Cells:
CHO, TE671

Tools:
Patchliner

Toxin block of $Na_V1.7$ and nAChR receptors on the Patchliner

Nanion Technologies GmbH, Munich
Alomone Labs

Summary

Traditional drug development for ion channels has predominantly focused on small molecules. While effective, this approach often encounters challenges, particularly with selectivity and safety. Biologics, including toxins and venom-derived peptides, have emerged as a promising alternative to address these limitations. Animal toxins have long been invaluable in ion channel research, offering insights into their structure, function, and gating mechanisms. Venoms are now recognized as a rich resource for innovative research tools and therapeutic leads. Peptide-based drugs, derived from these venoms, have gained attention for their unique advantages, such as larger interaction surfaces that confer greater specificity and selectivity compared to small molecules.

The study of toxins and venom-derived peptides has revolutionized our understanding of ion channels, uncovering new pathways for therapeutic development. Several venom-inspired ion channel targeting peptides have progressed to clinical development. For instance, Ziconotide, a synthetic ω-conotoxin peptide from cone snails, is used to treat pain by inhibiting the $Ca_V2.2$ channel and XEP-018, an $Na_V1.4$ blocker, inspired by cone snail μ-conotoxins, has found applications in cosmetics.

The advent of computational tools and the growing availability of ion channel crystal structures have made it increasingly feasible to design novel venom-inspired peptides with enhanced selectivity. High- and medium throughput automated patch clamp (APC) platforms are accelerating this process in industrial drug discovery and academic research alike.

Results

We tested toxins provided by Alomone Labs on $Na_V1.7$ expressed in CHO cells and nAChR $\alpha1\beta1\gamma\delta$ endogenously expressed in TE671 cells. $Na_V1.7$ currents were elicited using a single voltage step protocol to -10 mV. Current amplitude was stable over time when vehicle solution was added repetitively over the course of the experiment (approx. 10 mins; Fig. 1A). The toxins Huwentoxin-IV, TTX and GsMTx4 were tested on $Na_V1.7$. Huwentoxin-IV was added in increasing concentrations with 5 min incubation time per concentration. Huwentoxin-IV blocked $Na_V1.7$ with an IC_{50} of 54 ± 9 nM (n = 6; Fig. 1B), in good agreement with the literature[1,2,3]. TTX blocked $Na_V1.7$ with an IC_{50} of 27 ± 5 nM (n = 6; Fig. 1C) in good agreement with the literature[4,5]. GsMTx4 has been previously shown to block stretch activated[6] such as TRPC1[7], TRPC6[8] channels and mechanoactivated channels including Piezo1[9]. We found GsMTx4 blocks $Na_V1.7$ with an IC_{50} of 674 ± 58 nM (n = 10; Fig. 1D), a slightly higher potency than reported in the literature of 7.4 ± 0.3 μM (n = 4)[10].

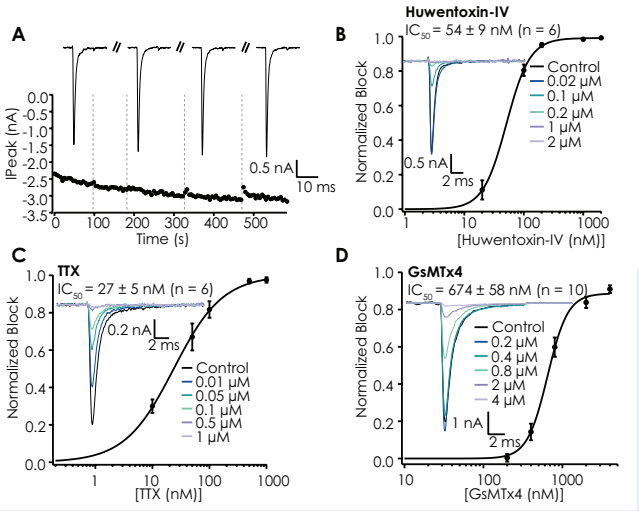

Figure 1: A *$Na_V1.7$ current amplitudes were highly stable over time when only vehicle was added to the cells.* **B** *CRC for Huwentoxin-IV block of $Na_V1.7$ for an average of 6 cells.* **C** *CRC for TTX block of $Na_V1.7$ for an average of 6 cells.* **D** *CRC for GsmTx4 block of $Na_V1.7$ for an average of 10 cells.*

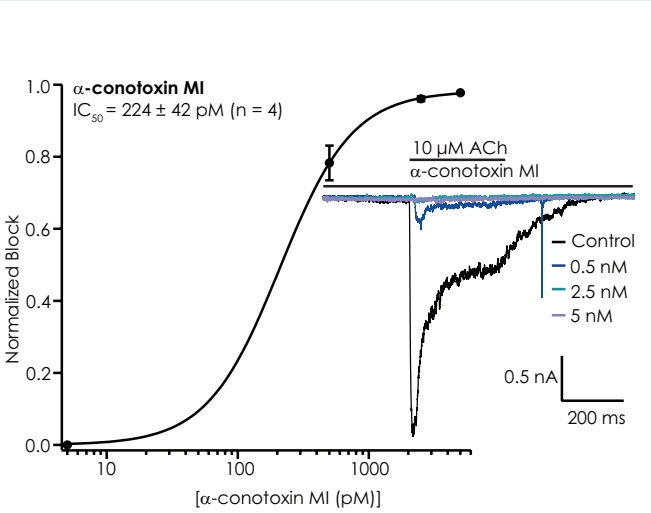

Figure 2: *α-conotoxin MI blocked nAChR expressed in TE671 cells with high potency. Shown is the CRC for α-conotoxin MI and the corresponding traces from an example cell.*

We also tested α-conotoxin MI from the venom of the *Conus magus* cone snail. We found that α-conotoxin MI blocked muscle-like nicotinic acetylcholine receptors (nAChR; subunit combination $\alpha1\beta1\gamma\delta$) expressed in TE671 cells with sub-nanomolar potency (Figure 2), in good agreement with the literature[11]. nAChR were activated using 10 µM acetylcholine (ACh) and the toxin was pre-incubated for 300 s before co-application with ACh. If the toxin was not pre-incubated the IC_{50} was 10X higher (2.5 nM; data not shown).

In summary, the Patchliner is an ideal tool to study effects of toxins on voltage- or ligand-gated ion channels. Small volumes, precise timing of solution addition and high success rates ensure efficient investigation of toxin potency and mode of action for various ion channels on the Patchliner.

References

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Methods

Cells

CHO cells stably expressing $Na_V1.7$ (Anaxon) or TE671 cells endogenously expressing nAChR $\alpha1\beta1\gamma\delta$ (CLS; #300355) were used.

Electrophysiology measurements

Cells were cultured and harvested according to Nanion's standard protocols. Cells were resuspended in Nanion's external recording solution (08 3001) and stored in the CellHotel of the Patchliner before being dispensed into each well of the NPC-16 chip. Nanion internal (08 3008) and external solution (08 3001 or 08 3004) compositions are available upon request. Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Patchliner. $Na_V1.7$ currents were elicited using a single voltage step protocol to -10 mV for 10 ms from a holding potential of -80 mV, repeated every 5 s. Toxins were kindly provided by Alomone Labs as stock solutions which were diluted to the final concentrations in external solution. Incubation time for TTX was 90 s, for GsMTx4 was 130 s and for Huwentoxin was 300 s per concentration. Toxins were added cumulatively on each cell in increasing concentrations. To activate nAChR, 10 µM was applied using the stacked solution approach and rapidly applied to the cells at a constant holding potential of -70 mV. α-conotoxin MI was preincubated for 300 s before co-application with 10 µM ACh. Data was analyzed using Igor (wavemetrics).