

Mechanistic screening for voltage-gated ion channel modulators

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Discovery Application Note

Introduction

Voltage-gated ion channels are complex gating proteins that govern the movement of ions across cell membranes. The time-course and voltage-dependence of gating transitions between closed (non-conducting), open (conducting) and inactivated conformational states of the channel are critical not only to physiological function, but also to modulation by drugs and biologics. As such, the ability to assay for modulators using pertinent voltage-command protocols and high temporal (kinetic) resolution is highly desirable. Historically, this could only be achieved using conventional patch clamp electrophysiology, which is low throughput and technically demanding. More recently, higher throughput automated electrophysiology methods, such as the IonWorks Quattro, provide the opportunity to do so at a speed and cost commensurate with hit identification and lead optimization screening. In this short article, we exemplify how valuable mechanism of action protocols can be introduced into early screens and used to quantitate and mechanistically differentiate compound classes.

Approach and Results

A stable cell line expressing the human voltage-gated Na⁺ channel Na_v1.7 was generated by Essen Bioscience in collaboration with Origene. HEK293 cells were transfected with Na_v1.7 cDNA and clones with resistance to an antibiotic marker were assessed for expression using the IonWorks platform. The preferred clone was functionally validated using a range of voltage-protocols (Figure 1). The basic methodology was similar to that described in Dale *et al*, 2007. Depolarising voltage-steps elicited characteristic, rapid, transient inward currents. Boltzmann analysis of the voltage-dependence of activation and inactivation yielded V_{1/2} values of -8 mV and -60 mV, respectively, close to those described previously (Klugbauer *et al*, 1995). The notion of 'use-dependent' Na⁺ channel inhibition as a desirable therapeutic mechanism has been established for a number of years (Ragsdale *et al*, 1994).

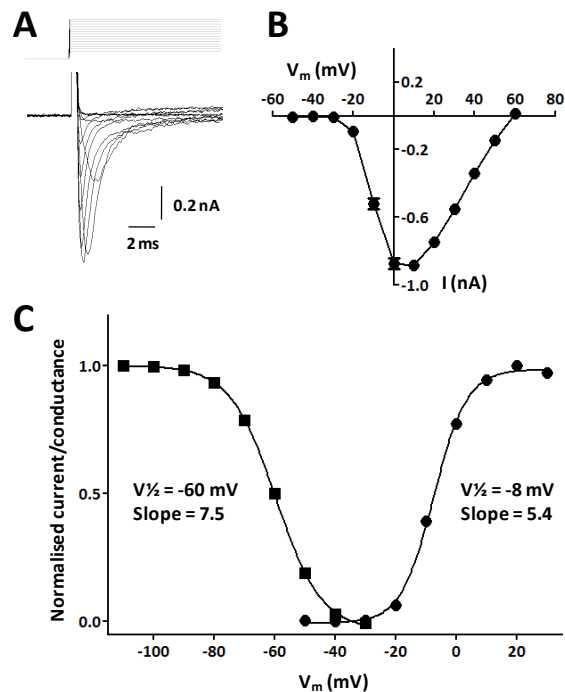


Figure 1. Biophysical characterization of Na_v1.7 channels expressed in HEK293 cells. **A)** Representative currents obtained by a family of depolarising pulses between -50 and +60 mV from a holding potential of -90 mV. **B)** Peak I-V relationship (mean ± SEM, n= 67). **C)** Normalised G (conductance)-V plot. To ensure appropriate voltage-clamp only cells with peak current amplitudes ranging from 0.4 to 1.2 nA were included. The reversal potential was +61 mV, close to the theoretical value of +66 mV, calculated using the Nernst equation. From the G-V plot the Boltzmann parameters for activation were: V_{1/2} -8 mV, slope of 5.4 (mV/e-fold). The voltage-dependence of inactivation was determined by applying 1 s conditioning pulses prior to a test pulse to 0 mV. From the inactivation plot the Boltzmann parameters for inactivation were: V_{1/2} -60 mV, slope of 7.5 (mV/e-fold)

Repeated channel gating leads to cycling of the channels through closed, open and inactivated states, thus allowing compounds that preferentially bind to certain states to produce enhanced blockade during the gating train. Classical, clinically-used, Na⁺ channel blockers such as tetracaine, lidocaine and lamotrigine act via this mechanism and their selective inhibition for open and inactivated states is pivotal to their therapeutic index. In contrast, the poisonous *Fugu* extract tetrodotoxin does not exhibit use-dependence (Figure 2A). To aid the hunt for novel use-dependent Na_v1.7 channel blockers we configured a 384-well IonWorks population patch clamp assay readily amenable to screening compound sets up to 50K in size. The fidelity of the assay is such that even subtle (<20%) use-dependent effects can be detected at a single assay concentration (Figure 2B). Incorporating this type of voltage protocol into hit finding phases of a

discovery program allows the identification and optimization of molecules with the desired mechanism of action.

However, this is not the full story. Whilst the use-dependent *in vitro* screening paradigms are designed to mimic the repeat firing patterns of peripheral neurons, it is difficult to truly model the complex interplay between channel states that occurs *in vivo*. Recently, Large *et al.* (2009) indicated that the (functionally determined) affinity of the compound for the inactivated state (K_1) of the Na^+ channel, provided the best correlate between *in vitro* and *in vivo* efficacy. The K_1 can be derived from the shift in the voltage-dependence of inactivation (K_{uo} & Bean, 1994). Thus, we configured a 384-well assay to measure the $V_{1/2}$ of inactivation in the presence and absence of compounds (Figure 3) as an improved ‘translational’ assay.

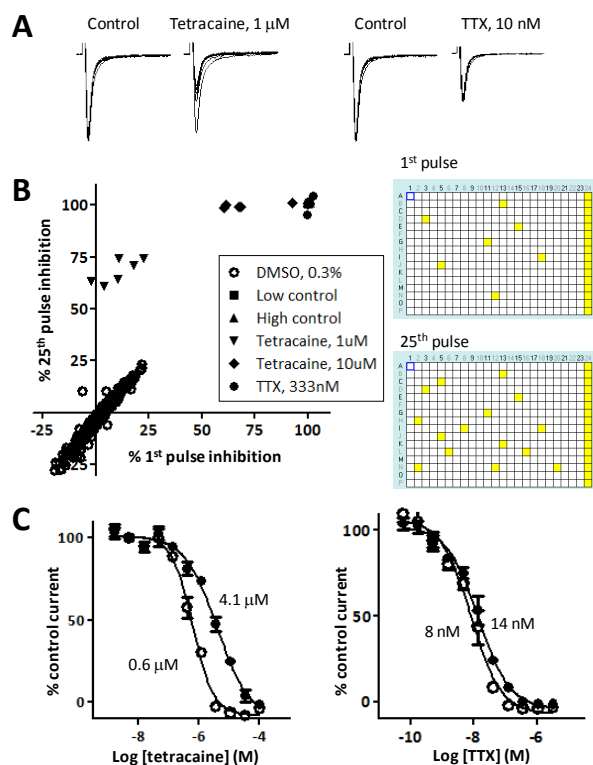


Figure 2. Use-dependent mechanism of action. A) Repeated depolarisations (V_H -80 mV, test pulse 0 mV, 20 ms, 10 Hz) applied in the absence (control) or presence of tetracaine (1 μM) or TTX (10 nM). B) Single concentration testing of a single compound plate containing vehicle (DMSO) and spiked wells (tetracaine 1 or 10 μM). The high and low controls were applied; TTX (3 μM) and DMSO (0.3%). 384-well plate views of the 1st or 25th pulse. Yellow wells represent >70% inhibition. C) Concentration-response curves for tetracaine and TTX using the 1st or 25th pulse metric.

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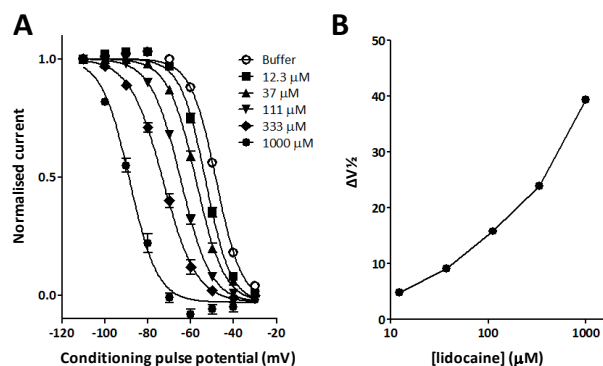


Figure 3. Shifts in inactivation. A) Inactivation curves constructed in increasing concentrations of lidocaine. B) Lidocaine produces a concentration-dependent shift in $V_{1/2}$ of inactivation, from which the K_1 can be determined.

Conclusion

The mechanism of action (MoA) of voltage-gated ion channel modulators has long been recognized as a critical component of their clinical profile. With care, mechanistic parameters can be derived in automated electrophysiology studies at a throughput commensurate with both hit identification and lead optimization SAR screening. Such ‘mechanistic screening’ should be invaluable in identifying novel ion channel modulators blockers with improved efficacy and safety profiles.

References

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