DEVELOPMENT OF A Na\textsubscript{V1.5} ASSAY FOR PROLONGED RECORDING ON AN AUTOMATED ELECTROPHYSIOLOGY SYSTEM

Edward Verdonk, Trisha Mitlo, Peter Miu, Craig McKay, James Costantin, Xin Jiang
Molecular Devices, Inc., 1311 Orleans Drive, Sunnyvale, CA 94089

Introduction

The IonWorks Barracuda system allows simultaneous and continuous measurement of both ligand-gated ion channels (LGICs) and voltage-gated ion channels (VGICs) in 384 individual recording sites. To achieve this, IonWorks Barracuda system is equipped with 384 individual patch-clamp amplifiers together with a 384-channel fluidic pipette. Similar to its predecessor, IonWorks Quatro, IonWorks Barracuda system measures cell membrane currents using the perforated patch clamp techniques on a polystyrene substrate. CURRENTS are measured using a single spike at each recording site or an array of 64 holes at each site (Population Patch Clamp or PPC, Friel et al., 2010). In this study, we evaluated a new technique to further prolong the assay window for ionic currents on the IonWorks Barracuda instrument. Using Nav1.1 channel as an example, we demonstrated stable recording of Nav1.5 currents elicited by repetitive scans for 30 minutes or longer. This prolonged assay window, validated using multiple recording parameters, is about twice of that of IonWorks Quatro. This allows the researchers not only to precisely control the state of the channel by voltage clamp, but also to potentially analyze molecules with slow binding rate to the channel. To validate the suitability of this approach in a drug screening, here we designed and validated a single blind assay to first screen and then confirm use and non-use-dependent blockers of Nav1.5 channels. The high throughput, high sensitivity and the robustness of IonWorks Barracuda system makes it an ideal platform in screening use-dependent channel blockers in a drug discovery environment.

Material and Methods

Cells: Chinese hamster kidney (CHK) cells stably expressing the human SCN5A (Nav1.5) gene.
Reagents and buffers: Amphoterin (Sigma Cat. # 4-A4888), DMSO (Sigma Cat. # D-2651); Internal buffer contains (mM): 100 KCl, 3.2 MgCl\textsubscript{2}, 5.0 Hepes, pH 7.25 with 100mM External buffer is Phosphate Buffered Saline (PBS, Gibco Cat. # 14040).

Electrophysiology: All experiments were performed in the PPC mode, with voltage-clamp, and compound addition protocols described in the figure. Data analysis: In addition to the native filter, two data filtering criteria were implemented, 1) seal resistance < 50 M\textOmega, and 2) seal resistance change < 50% (pre vs. post). The data were analyzed and plotted using Prism 5 software.

Biophysical characterization of Nav1.5 channels

Fig. 2. The voltage-dependent activation and voltage dependence of inactivation of Nav1.5 channels were examined on the IonWorks Barracuda system, in the PPC mode. A) B) and C) represent representative Nav1.5 currents elicited from well (top traces), using different voltage protocols (bottom). C) and D) the current-voltage relationships for both activation and inactivation of Nav1.5 channels, data was collected from one experiment (mean ± SEM, n= 382 wells, 2 wells filtered out).

Validation of Nav1.5 screening protocol

Fig. 3. To screen for use and non-use dependent compounds, a train protocol was designed and validated based on the biophysical properties of the channel. A) Top: diagram of the voltage protocol, Vh = -100mV, test voltage = +35mV for 20ms. Middle: representative Nav1.5 currents elicited from one well in PPC mode; Bottom: zoom-in view of the currents elicited by the first (P1) and last (P30) pulses.

Descriptive analysis of the screening process: After baseline measurement, in each assay 10µl of compound (1X concentration) or buffer were introduced into PatchPlate well at medium position, with no mixing. Three post-compound scans were collected at 0s, 90s, and 180s after compound addition. C) The current amplitudes of test pulse #1 and #30, for all scans, were examined by repeated measures one-way ANOVA with Tukey's post hoc test for between group comparison among all groups, indicating the stability of currents over time, and after compound (buffer in this case) addition.

Validation of prolonged assay window

Fig. 4. Evaluation of assay window for Nav1.5 channels, and recording stabilities among multiple experiments. A) Potassium currents were elicited in 384 compound plate, with a buffer addition 3 minutes into each experiment. B) peak current stability with the cells subjected to 4 repetitions of the screening protocol (n= 16).

Concentration-response analysis for hit confirmation

Fig. 6. Pharmacological characterization of concentration-response relationship, for confirmation of screening hits. A), plate view of Nav1.5 currents (pulse 30 only) in response to different concentrations of compounds (TTX, lidocaine, tetracaine) or buffer; B), heat map view of the wells with more than 50% inhibition of pulse 30 currents; C), representative currents (top) and the overlay of pre- and post-compound currents (bottom) from the same well.

Concentration-response analysis for hit confirmation

Fig. 7. Identification of use-dependent compounds by comparing concentration-response curve. The data were log-transformed (A) and pulse 30 (B), and at different time points. C) data scatter from three different experiment, indicating highly consistent results.

Summary

In this study we designed and validated a high-throughput electrophysiology assay for screening and confirming blockers of a voltage-gated sodium channel. The pharmacology data collected on IonWorks Barracuda system is in good agreement with literature reports.

The IonWorks Barracuda system enables continuous voltage clamp, sophisticated voltage protocols, flexible recording parameters, and prolonged assay window, for identification of use-dependent compounds in a drug screening setting.