Assay of ASIC1a Channel with the IonWorks Barracuda System

By Xin Jiang, Ph.D., Ed. Verdonk, Ph.D. Trisha Tutana and James L. Costantin, Ph.D., Molecular Devices, Inc. 1311 Orleans Drive, Sunnyvale, CA 94089.

Introduction

Ligand-gated ion channels (LGICs) represent an important class of drug targets for a wide range of neurological and psychiatric diseases. Despite the great interest in screening compounds for LGICs, the existing technologies are limited. Surrogate high-throughput assays such as fluorescent or ion flux assays lack voltage control, and the data acquisition is often too slow to analyze fast-desensitizing LGICs. On the other hand, direct electrophysiological assays measure real-time change of channel activity with highest data quality; however they are either low-throughput, or cost-prohibiting, even for existing automated electrophysiology systems.

To address the unmet demand for a high-throughput, low-cost electrophysiology system for assaying LGICs, we developed the IonWorks Barracuda™ Automated Patch Clamp System, which records currents continuously and simultaneously from 384 parallel recording sites and delivers a throughput of > 1,100 data points per hour. In this study, we present an assay of acid-sensing ion channels (ASICs) using the IonWorks Barracuda System. ASICs are ligand-gated cation channels which act as extracellular pH sensors in peripheral sensory neurons. Upon the binding of protons, ASICs open quickly and then rapidly enter a desensitized state where the channel closes on a time scale of seconds. By demonstrating that the IonWorks Barracuda System can reliably record ASIC1a currents with proper electrophysiological and pharmacological profiles we have shown how the system can be used to assay ligand-gated ion channels.

Methods and Results

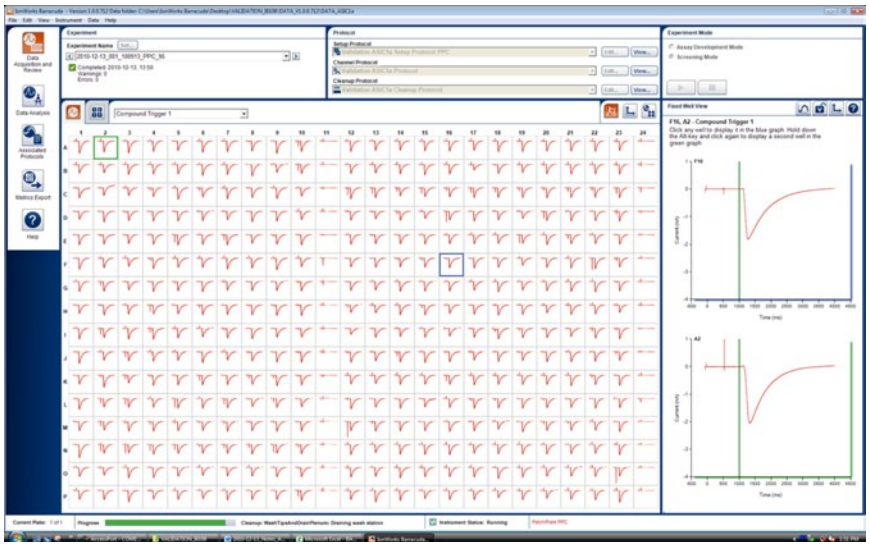
Chinese hamster ovary (CHO) cells stably transfected with human ASIC1a ion channel were provided by ChanTest® Corporation (Cat. #CT-6012, Cleveland, OH). For electrophysiological recordings on the IonWorks Barracuda System the cells were lifted with accutase. The ASIC1a currents are elicited by rapidly replacing the regular external buffer (pH = 7.3) with acidic buffer (pH = 5).

Recording of ASIC1a currents on the IonWorks Barracuda System

The IonWorks Barracuda System is equipped with 384 parallel amplifiers with each recording site controlled by one dedicated patch clamp amplifier. The recording electrodes remain inserted in respective wells for the duration of the experiment so to maintain continuous voltage clamp and, when required, perform simultaneous recording during the compound addition. Each recording site is serviced by a dedicated pipettor tip to maximize the throughput and prevent cross-well contamination. Similar to the IonWorks® Quattro Automated Patch Clamp System, the IonWorks Barracuda System operates in both single hole (SH) or population patch

clamp (PPC) modes, where currents from either one cell (SH) or 64 cells (PPC) are acquired per well. In this study, the cells were held at -70 mV for 4 seconds; and the currents continuously recorded during addition of ligand (low pH buffer) introduced after the first second (Figures 1 and 4).

ASIC1a Currents on IonWorks Barracuda System (Figure 1)

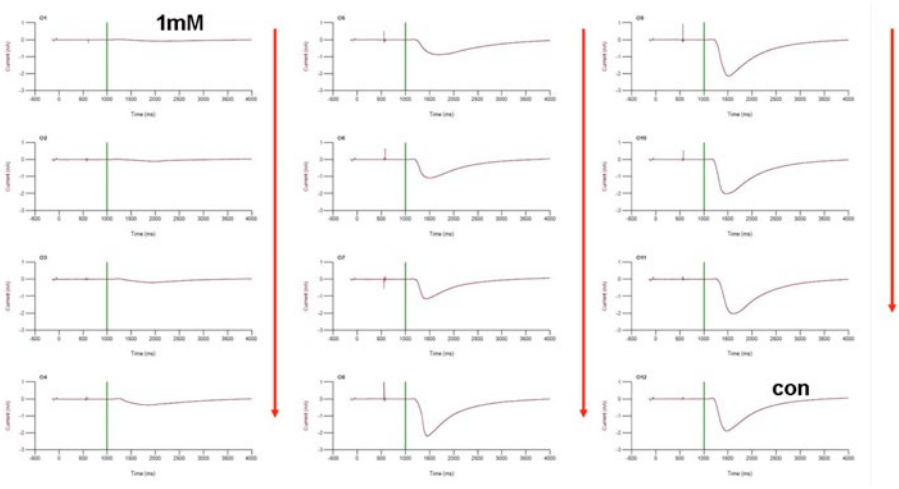


Plateview of whole-cell ASIC1a currents obtained from the IonWorks Barracuda System. The experiment was performed in the PPC mode. Columns 12 and 24 were challenged with external buffer only as negative control. Note the successful recording of expected signals in all 384 recording sites (100% success rate) as well as the uniformity of ASIC1a currents across the plate, indicating the variation of channel expression commonly seen in the SH mode is greatly mitigated.

High uniformity of signals in PPC mode enables cross-plate comparison for pharmacological assays

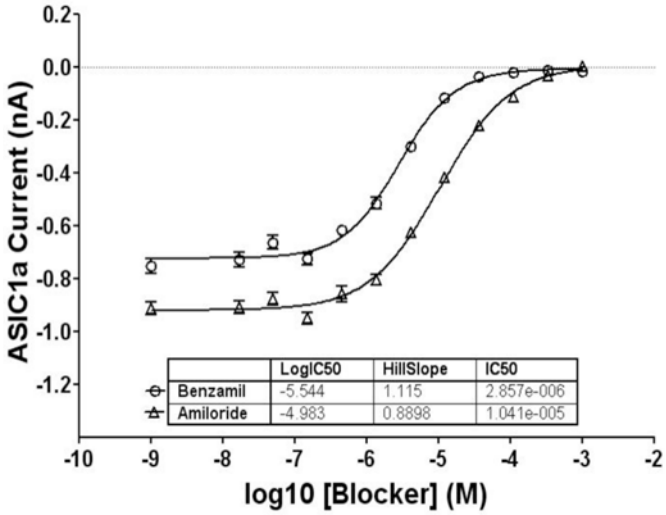
Population Patch-Clamp™, introduced in 2005 by Molecular Devices, records ensemble currents from cells sealed onto 64 recording aperatures using a single amplifier per well. PPC mitigates the variability inherent to conventional and single aperture planar patch-clamp techniques caused by variability of exogenous channel expression, low seal resistances, and unstable recordings. The use of PPC on the IonWorks Barracuda System not only improves the success rate, *i.e.* the acquisition of a data point from each recording site, to nearly 100%; but also greatly enhances the uniformity and consistency of signals across the PatchPlate™ Consumable, allowing cross-well comparisons for pharmacological assays (Figures 2 and 3). In doing so, the user can simply compare the change of currents to neighboring recording sites without worrying about the incomplete washout or channel rundown associated with the conventional approach of repetitive compound/ligand application and washout. In addition, this greatly shortens the assay time since only one compound addition is performed at each recording site. The high success rate in the PPC mode, combined with fast assay time (typically 20 min/assay), allow an unparalleled throughput of >1,100 data points per hour.

Blockade of ASIC1a currents by Amiloride in PPC mode (Figure 2)



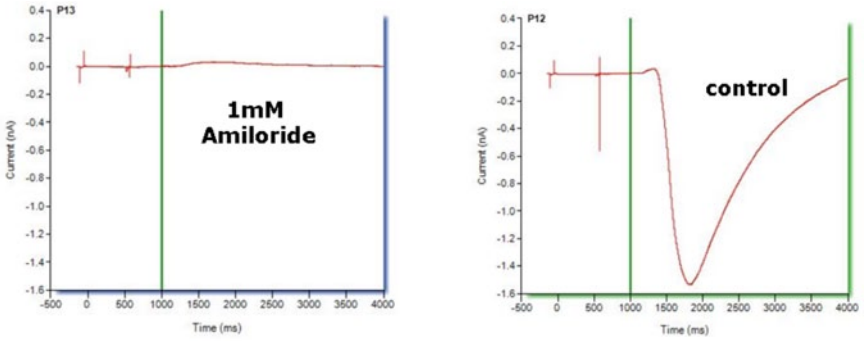
Cross-well comparison in the PPC mode demonstrates concentration-dependent inhibition of ASIC1a currents by amiloride. In this PPC experiment, 11 decreasing concentrations of amiloride (1:3 dilution from 1 mM in the well displayed at the upper left to 17 nM as indicated by the vertical arrows) were first applied to cells in different wells before low pH buffer was introduced to elicit ASIC currents. The amplitude of ASIC currents increases progressively as the concentration of amiloride decreases; the well at the lower right is a buffer control.

CHO-ASIC1a channel blockers (Figure 3)



Concentration-dependent inhibition of ASIC1a currents by amiloride and benzamil. The data was collected from two different PPC experiments where the absolute ASIC1a currents obtained with 11 different concentrations of blockers were pooled and plotted. The IC_{50} values match those reported (ChanTest Product Data Sheet for Cat# CT6012; 2010). Each data point represents 25-32 wells.

ASIC1a currents in the presence and absence of amiloride (Figure 4)



Representative recordings of ASIC1a currents in the presence and absence of 1 mM amiloride. In both panels the green bar indicates the time ligand (low pH buffer) is delivered into the PatchPlate Consumable. Note the current spike that occurs prior to the green bar is an electrical artifact due to pipettor tip touching the solution.

Summary

The data presented here demonstrates the suitability of the IonWorks Barracuda System to assay ligand-gated ion channels. Continuous recording during ligand addition in 384 parallel recording sites, in addition to nearly 100% success rate in the PPC mode, provide the IonWorks Barracuda System with unparalleled throughput. A 384-well compound plate can be assayed in 20 minutes yielding a throughput of over 1,100 data points per hour. The high throughput combined with the lowest running costs per data point make the IonWorks Barracuda System the best option to screen both voltage- and rapidly desensitizing ligand-gated ion channels.

References

1. Pidoplichko VI and Dani JA. (2006) Acid-sensitive ionic channels in midbrain dopamine neurons are sensitive to ammonium, which may contribute to hyperammonemia damage. *Proc Natl Acad Sci USA* 103(30):11376-80

2. Lingueglia E. (2007) Acid-sensing ion channels in sensory perception. *J Biol Chem* 282(24):17325-9

Molecular Devices

Contact Us
Phone: +1-800-635-5577
Web: www.moleculardevices.com
Email: info@moldev.com
Check our web site for a current listing of worldwide distributors.

Regional Offices
USA and Canada +1-800-635-5577
Brazil +55-11-3616-6607
China (Beijing) +86-10-6410-8669
China (Shanghai) +86-21-3372-1088
Germany +49-89/96-05-88-0
Japan (Osaka) +81-6-6399-8211
Japan (Tokyo) +81-3-5282-5261
South Korea +82-2-3471-9531
United Kingdom +44-118-944-8000