Validation of TRPV1 and ASIC1 ligand-gated ion channels using automated patch clamp and FLIPR with novel Ca\textsuperscript{2+} detection dye

Carole Crittenden\textsuperscript{1}, Xiaoyi Du\textsuperscript{2}, Yuri Kuryshev\textsuperscript{2}, Emir Dulcic\textsuperscript{2}, and Xin Jiang\textsuperscript{1}

\textsuperscript{1} Molecular Devices, LLC, 1131 Orleans Drive, Sunnyvale, CA 94089
\textsuperscript{2} ChanTest Corporation, 14656 Neo Parkway, Cleveland, OH 44128

Introduction

Ligand gated ion channels (LGICs) are a large family of membrane embedded proteins that enable the passage of ions across membranes, in response to the binding of ligands such as neurotransmitters. LGICs represent a class of highly attractive drug targets because of the pivotal role they play in many physiological functions, and their association with multiple human diseases. Among existing ion channel screening technologies, the fluorescent probes, especially the calcium sensing dye, have become indispensable tools for analyzing ion channel activities. The simplicity of the assay, the high throughput, as well as the low turn around cost, makes this method especially attractive to large scale primary screening in the early phase of the drug discovery process. In an attempt to further improve such assay quality we evaluated the use of a novel calcium-sensing dye in LGIC assays. The unique molecular configuration of this dye, combined with a novel quenching technology, provides distinctive advantages over existing calcium probes by (1) providing a larger signal/noise ratio, and (2) removing the need of probendazol in the assay. Specifically, in this study we examined two calcium permeable ligand gated ion channels: acid sensing ion channel 1a (ASIC1a) and transient receptor potential vanilloid 1 (TRPV1).

Results

**Ligand gated ion channels with calcium sensitive dye**

**ASIC1a Channel**

The ASIC1a Channel was successfully characterized for the FLIPR Calcium 6 dye selectivity and toxicity. The absolute current amplitude was reduced by ~60% with calcium removed from the external buffer (A, C), whereas the membrane resistance (D), the gating of the channel and the profile of the currents appears unaffected, suggesting that the ASIC1a channels functions normally in the absence of external calcium. In the second set of experiment the cells were pre-incubated with Ca\textsuperscript{2+} dye for 2 hours before being subjected to electrophysiological recording on the IonWorks Barracuda system. The amplitude of channel current was unaffected (B, C) despite a drop in membrane resistance (D), suggesting the Ca\textsuperscript{2+} dye doesn't affect the channel properties within the tested time frame.

**Discussion and conclusions**

1. We have validated the utility of a novel calcium sensitive dye for sensitive, high-throughput, fluorometric assays of two ligand-gated ion channels.
2. The data presented here indicates this novel dye is selective for calcium ions. Under the experimental conditions described in this study, the dye itself does not alter the biophysical properties of the channels.

**References**