Label-Free Ion Channel Assays Using the Corning® Epic® System

SnAPPShots

A brief technical report from the Corning Development Group

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Introduction

Ion channels comprise one of the most important classes of drug targets currently under therapeutic investigation. Traditional electrophysiology assays using the patch clamp technique record the flow of electrical current across a single channel but are not readily incorporated into high-throughput screening (HTS) platforms. In contrast, ion flux assays that measure rapid changes in membrane potential can be run in highly automated HTS configurations containing standard microplate readers. However, a major drawback of this approach is that these assays rely on the use of voltage-sensitive dyes, which are likely to interfere with the normal physiology of the cells under investigation, and may be toxic at high doses (1).

The Corning Epic System is a high-throughput label-free screening platform that provides a new approach to studying ion channels. High sensitivity optical biosensors measure a kinetic profile of the dynamic mass redistribution (DMR) that occurs following modulation of channel activity. Label-free ion channel assays do not require toxic dyes; therefore ion channel activity can be assessed in a more physiologically relevent setting. Furthermore, response profiles are highly amenable to HTS, offering an alternative approach to the screening of compounds that alter ion channel behavior.

As a model system for evaluating ion channel responses on the Epic System, HEK293 cells, engineered to overexpress the $\alpha1\beta2\gamma2$ $GABA_A$ ion channel (Wss-1 cells), were used. Studies of the $GABA_A$ ion channel have resulted in identification of several agonist and antagonist compounds that have been extensively characterized using conventional electrophysiology approaches. Here it is demonstrated that the Epic System can be used to measure DMR responses in Wss-1 cells using these compounds. Pharmacology data obtained using the Epic System was in accordance with values previously reported using patch clamp and ion flux assays. Robustness of label-free assays was demonstrated in whole 384 well microplate assays measuring the response to a fixed dose of agonist. These studies demonstrate the utility of the Epic System for cell-based HTS ion channel assays.

Materials and Methods

Cells

Wss-1 cells (Cat. No. CRL-2029) were obtained from American Type Culture Collection (Manassas, VA) and maintained in Dulbecco's Modified Eagle Medium supplemented with 10% heat-inactivated Fetal Bovine Serum and 2 mM L-Glutamine. All cell culture reagents were purchased from Invitrogen® (Carlsbad, CA).

Reagents

GABA, Muscimol, Bicuculline and Gabazine were all purchased from Tocris Bioscience (Ellisville, MS). Additional agonist compounds, Isoguvacine, Gaboxadol and Piperidine-4-sulfonic acid were obtained from Sigma-Aldrich® (St. Louis, MO).

Epic Assay Procedures

Cell Seeding

Stock subcultures of Wss-1 cells were grown to ~80% confluency on standard tissue culture treated growth surfaces for 3 days, then harvested using trypsin. Harvested cells were diluted in complete growth medium and counted. Cells were then seeded into Epic 384 well Fibronectin-Coated Cell Assay Microplates (Corning Cat. No. 5042) using a Thermo Scientific® Multidrop® Combi cell culture dispensing system at a density of 10,000 cells/well. The seeded microplate was allowed to sit at room temperature for 30 minutes to allow the cells to uniformly settle on the growth surface of the well and then incubated prior to beginning the assay (average 18 to 22 hours in a 37°C/5% CO_2 incubator).

CORNING
Epic system

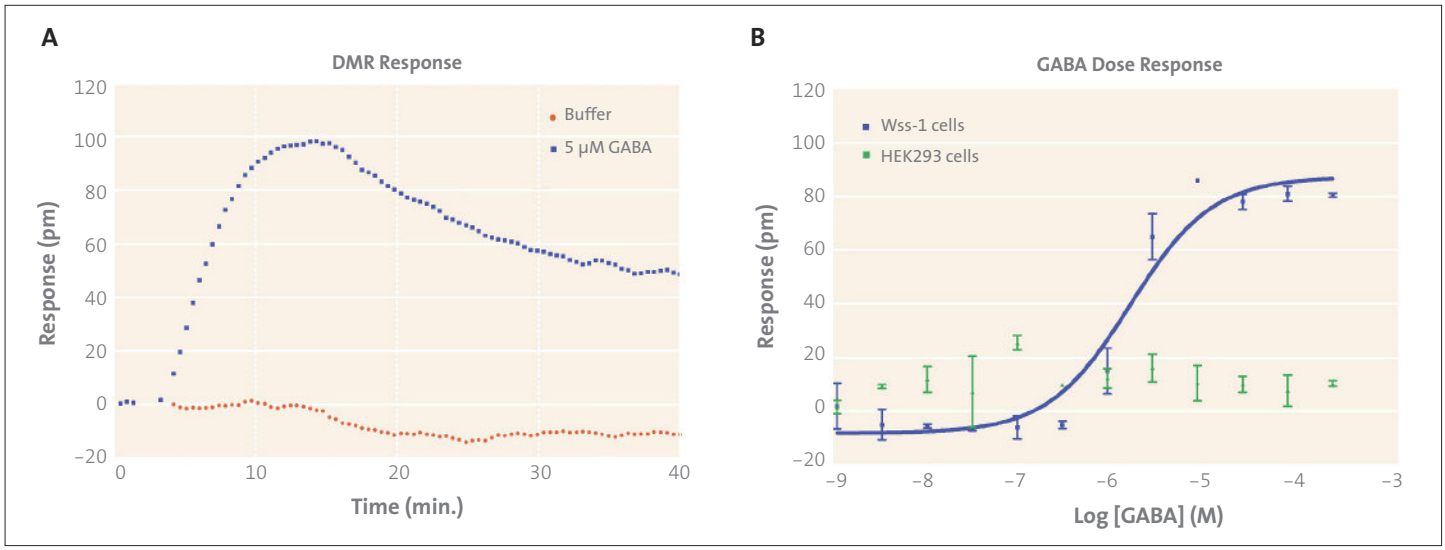


Figure 1. GABA stimulates a specific DMR response in Wss-1 cells. Addition of 5 µM GABA to Wss-1 cells produces a positive DMR response of ~100 pm that peaks between 10 and 20 minutes after compound addition (A). Dose response analysis demonstrates that the GABA response is specific to Wss-1 cells and fails to elicit any response in parental HEK293 cells (B).

Buffer Exchange

After overnight culture, the growth medium was replaced with assay buffer containing 1% DMSO using the Epic® Liquid Handling Accessory (LHA) pipetting system. The microplate was then stored on the carousel inside the Epic Reader for 1 to 2 hours prior to running the assay to allow for thermal equilibration of the cells.

Epic Measurement

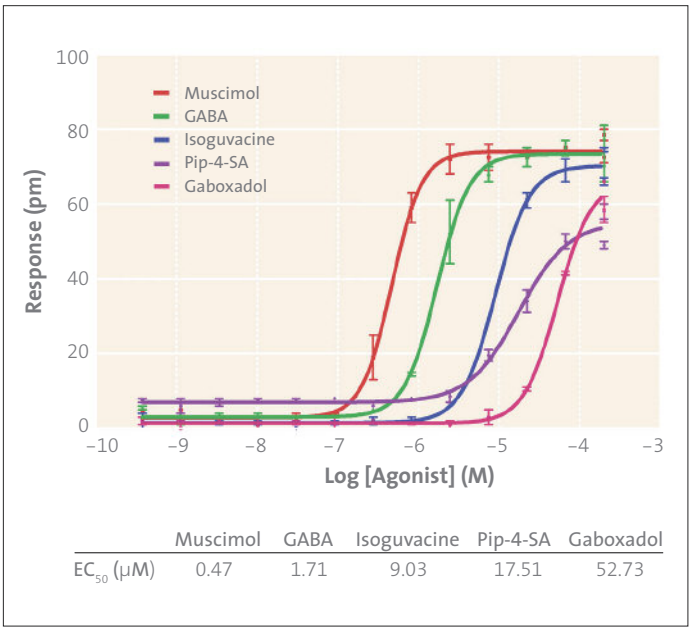
Following thermal equilibration, the assay microplate was transferred into the nest and a baseline measurement was taken for 2 minutes. The assay microplate was then exported from the reader for addition of compound using the LHA pipetting system. Finally, the assay microplate was returned to the nest in the reader and kinetic data profiles were measured for approximately 40 minutes. For antagonist experiments, the same procedure was used except that the antagonist and agonist compounds were added simultaneously to the microplate, and the kinetic profile of the agonist was then recorded.

Data Analysis

The kinetic profiles were analyzed using the Epic software and dose response curves were generated using GraphPad Prism® software. For each compound, the difference between the last baseline measurement and the peak response following compound addition was calculated.

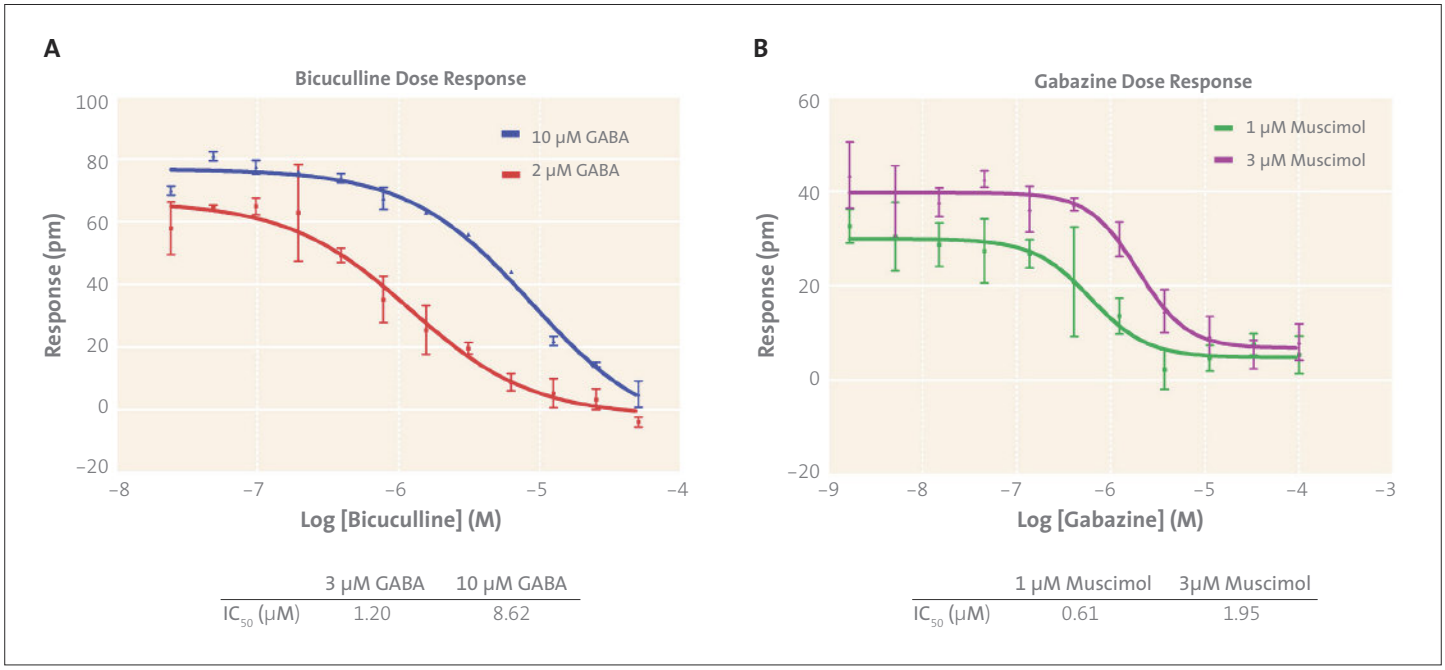
Results and Discussion

In order to determine whether the Epic System could be used to detect $GABA_A$-specific responses, GABA was added to Wss-1 cells and the DMR responses were monitored. Compound addition resulted in a distinctive DMR response with an initial positive DMR phase that peaked between 10 and 15 minutes, and then transitioned into a slower negative DMR phase which eventually reached a plateau by 40 minutes (Figure 1). Using the peak response to measure activity, a dose response series was tested in Wss-1 cells and the parental HEK293 cells. Figure 1 shows that the GABA response in Wss-1 cells was dose-dependent and saturable with an EC_{50} value of 1 to 2 µM, which is in very good



	Muscimol	GABA	Isoguvacine	Pip-4-SA	Gaboxadol
EC_{50} (µM)	0.47	1.71	9.03	17.51	52.73

Figure 2. Agonist rank order of potency can be determined on the Epic System. Dose response analysis was performed in Wss-1 cells with several well characterized $GABA_A$ agonist compounds and the resulting EC_{50} values were determined using Graphpad Prism software.



	3 µM GABA	10 µM GABA
IC_{50} (µM)	1.20	8.62

	1 µM Muscimol	3µM Muscimol
IC_{50} (µM)	0.61	1.95

Figure 3. Antagonists dose-dependently inhibit the GABA response in Wss-1 cells. Addition of either GABA (A) or Muscimol (B) at EC_{60} and EC_{90} doses to Wss-1 cells in the presence of known $GABA_A$ antagonists results in a dose-dependent inhibition of the agonist response.

agreement with literature values using ion flux assays (2). In contrast, no response was observed in the HEK293 cells, even at doses of 100 µM. Consequently, the DMR response measured on the Epic® System is highly specific to Wss-1 cells.

The Epic System can be used to differentiate compounds of varying potency. A panel of well characterized $GABA_A$ agonists for dose response analysis was selected, and the rank order of potency at the peak response (Figure 2) was determined. Each of the agonists resulted in a DMR profile similar to GABA (Figure 1), with the peak response occurring at the same time point. These data are in excellent agreement with electrophysiology studies using *Xenopus laevis* oocytes engineered to express the same $GABA_A$ receptor subunit combination (3). Potency of antagonists was also studied in the Wss-1 cells. Bicuculline and Gabazine were selected as $GABA_A$ receptor antagonists, and were both able to dose-dependently inhibit the DMR response of either GABA or Muscimol (Figure 3). Chloride influx assays on neuronal cells isolated from the cerebral cortex of Sprague-Dawley rats previously revealed that Gabazine has a more potent antagonistic effect than bicuculline (4). The data on the Epic System with these same compounds confirm the potency order, using the test agonist at EC_{60} and EC_{90} doses (Figure 3).

Screening for compounds that modulate the activity of ion channels requires that assay conditions be highly robust. The robustness of $GABA_A$ assays on the Epic System was

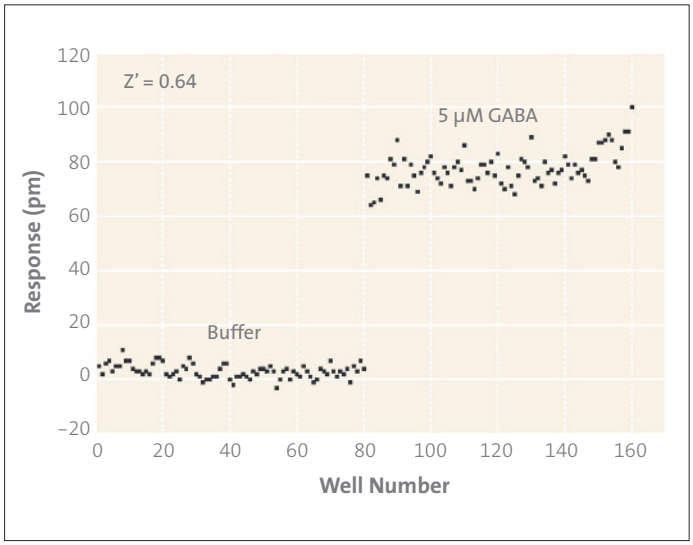


Figure 4. $GABA_A$ assays on the Epic System are very robust. Assay robustness was evaluated using an EC_{80} dose of GABA, and the peak response plotted using GraphPad Prism® software (n= 80 wells). The experiment was performed in triplicate and each time the Z' exceeded 0.6.

determined by adding an EC_{80} dose of GABA (5 µM) to Wss-1 cells and measuring the peak DMR response. Robustness assays were performed in triplicate, and in each case the Z' value for the assay exceeded 0.6 (Figure 4). These data establish that the Epic System can be used for HTS ion channel assays.

Conclusions

- Ion channel-specific DMR responses were observed on the Epic® System.
- Pharmacology of GABA response on the Epic System was in accordance with patch clamp and ion flux data.
- Rank order potency of several $GABA_A$ agonists and antagonists on the Epic System was in very good agreement with electrophysiology studies.
- Ion channel assays on the Epic System were very robust and amenable to screening in a label-free high-throughput format.

References

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