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APPNOTE

Recording of Endogenous $GABA_A$ Channels in Human Airway Smooth Muscle Cells

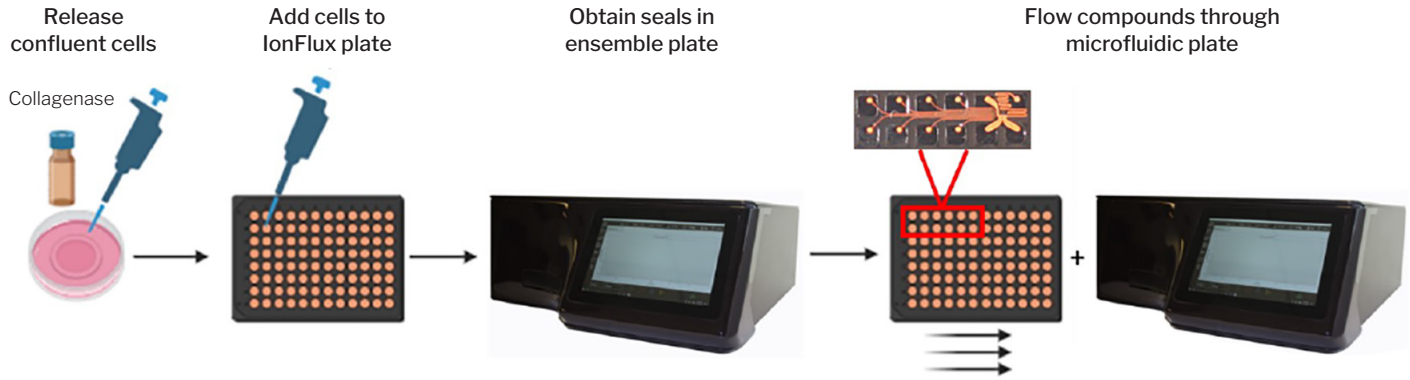
Introduction

Classical patch clamp assays typically use recombinant cell lines in which cells have been transfected to express the ion channel of interest. However, disparities between results from recombinant cells and primary cells have been observed, raising concerns about the physiological relevance of patch clamp assays of recombinant cells. Therefore, the ability to perform patch clamp assays for drug discovery and development on non-transfected primary cells is ideal. In this application note, we present high-quality automated patch clamp recordings from endogenous $GABA_A$ channels in human airway smooth muscle cells.

Key Highlights:

1) High-throughput assay of endogenous ion channels in non-transfected cells
2) Enhance the physiological relevance of automated patch clamp assays
3) Achieve patch clamp success rates for primary cells above 70%

Workflow



Questions this App Note Answers

1. How can endogenous ion channels be assessed using an automated patch clamp system?
2. Is the assessment of endogenous ion channels using an automated patch clamp system consistent and reproducible?
3. How does the assessment of endogenous ion channels compare with overexpressed cell lines?

Methods

Cells

Human bronchial smooth muscle cells were immortalized by stabilizing the expression of human telomerase reverse transcriptase (hTERT) 2. Stabilizing expression of hTERT2 extends the life span of smooth muscle cells but does not impact the expression of ion channels. Cells were grown to confluence in 15 cm dishes coated with 5 µg/cm^2 rat tail collagen type 1 (BD Biosciences). Cells were grown in M199 media with 10% fetal bovine serum, 0.25 ng/mL epidermal growth factor, 1 ng/mL fibroblast growth factor, ITS supplement (1 mg/mL insulin, 0.55 mg/mL transferrin, 0.67 µg/mL sodium selenium), and antibiotics (100 units/mL penicillin G sodium, 100 µg/mL streptomycin sulfate, 0.25 µg/mL amphotericin B) at 37°C, 5% CO_2.

Prior to testing, media was aspirated and 500 U/mL collagenase type IV (Sigma) was added. Dishes were incubated for 15 minutes at 37°C. Cells were released from the dish by gentle scraping with a pipette and cells were transferred to a 15 mL conical tube. Cells were centrifuged for 3 minutes, 300 x G at room temperature. The cell pellet was then resuspended in the desired external buffer solution and washed twice. Cells were resuspended at 3-5 x 10^6 cells/mL and loaded into the inlet wells of a 96-well IonFlux plate.

Ion Channel Recording

Cells were clamped at a holding potential of -80 mV. GABA was consistently applied for 3 seconds per dose, followed by a 30-60 second buffer wash to allow channels to return to baseline. GABA was applied at dosages of 10, 30, and 1000 µM.

To compare the performance of cells with endogenous ion channel expression to traditional overexpressing cells, fresh and frozen hGABA$_A$ (α1/β3/γ2) HEK cells were assayed in different wells of the same plate as the smooth muscle cells. Data analysis and graphical generation were performed using a combination of IonFlux software, Microsoft Excel, and Origin Lab. All data are presented as mean ± standard error of the mean (SEM).

Results

Average seal-resistances and success rates are presented in Figure 1. 12 of 16 traces showed detectable currents (>500 pA) (Figure 2A). In comparison to an overexpressing HEK cell line, success rates were lower for smooth muscle cells (98 vs. 75%). Seal-resistances for smooth muscle cells were lower when compared to fresh HEK cells (~100 vs. ~500 Mohm); however, seal-resistances for smooth muscle cells and frozen HEK cells were similar. The GABA dose-response for GABA$_A$ channels is presented in Figure 3. A dose-dependent response to GABA was observed with the largest current (~1.5 nA) being observed in response to the highest concentration of GABA.

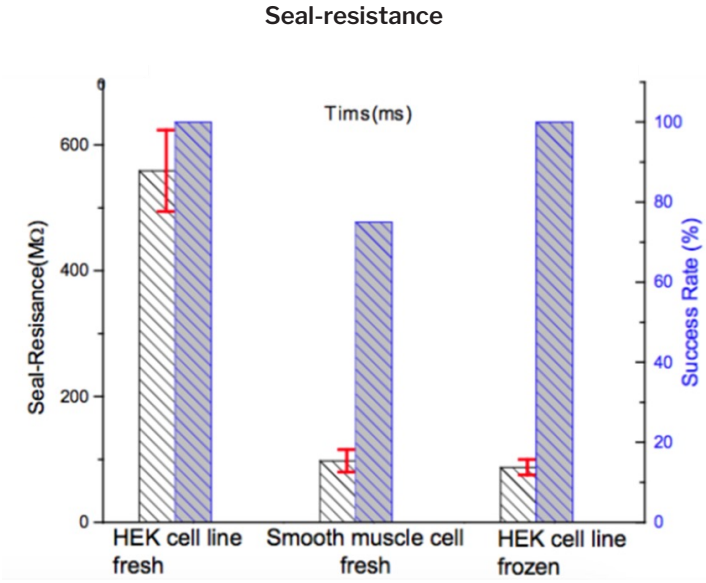


Figure 1. Average seal-resistance (Black and white bars) and success rates (Blue and grey bars) from smooth muscle cells, fresh HEK cells, and frozen HEK cells.

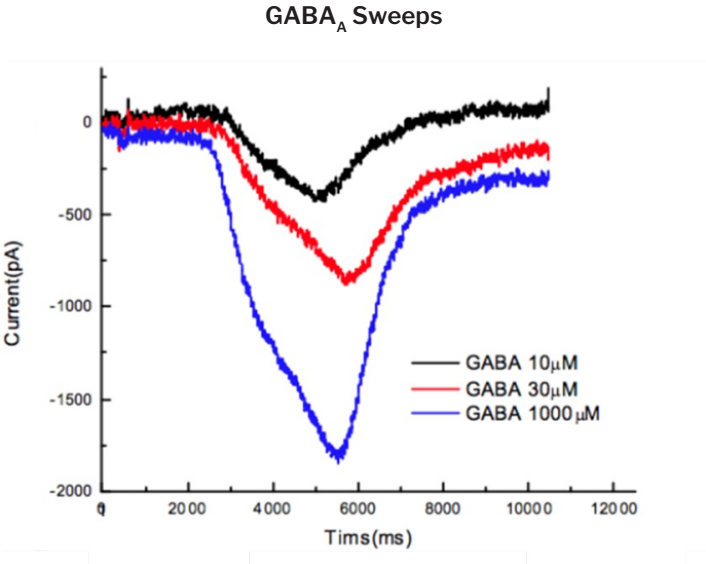


Figure 3. Endogenous GABA$_A$ channel recordings in response to increasing concentrations of GABA.

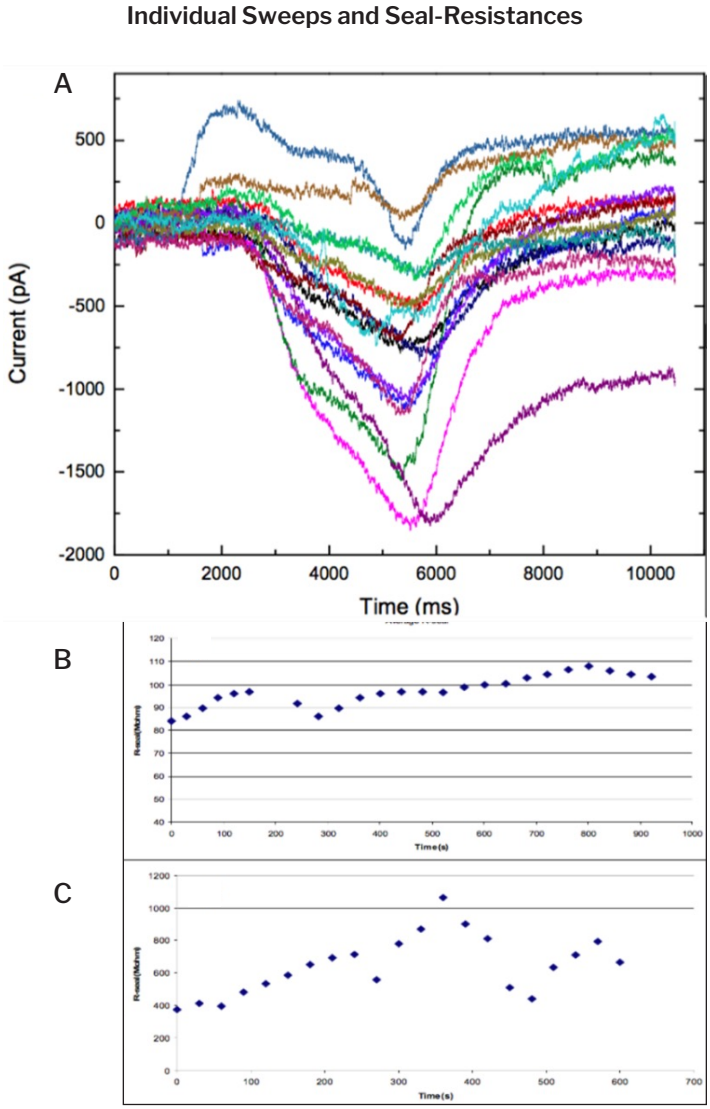


Figure 2. An IonFlux screenshot showing inward Cl^- current during the application of 1000 μM GABA to smooth muscle cells (A). Average seal-resistances for smooth muscle cells over the duration of the experiment (B). Average seal-resistances of fresh hGABA$_A$ (α1/β3/γ2) recombinant HEK cells over the duration of the experiment (C).

Discussion

Although manual patch clamp assays can be used to assess ion channel function, the process is highly technical and extremely low throughput, making manual patch clamp unfeasible for drug discovery and development. Therefore, many investigators have turned to automated electrophysiology assays to increase compound throughput. However, traditionally, automated systems have relied on transfected cell lines that overexpress the ion channel of interest. This has created a disconnect between *in vitro* results and the true *in vivo* response. To enhance the physiological relevance of automated patch clamp assays, it is necessary to test cells with endogenous ion channel expression.

In this report, we demonstrate the ability of the IonFlux automated patch clamp system to study endogenously expressed $GABA_A$ in immortalized human bronchial smooth muscle cells. Using a 20-cell ensemble plate with continuous liquid perfusion, we were able to achieve high success rates (75%) with good signal-to-noise ratios. These data are in agreement with previous manual patch clamp recordings, suggesting that this approach is well suited for physiologically relevant interrogation of non-transfected cells. Therefore, using an IonFlux system is a feasible method to enhance the throughput and physiological relevance of drug discovery and development investigations.

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