Recording of Endogenous $\text{GABA}_A$ Channel in Human Airway Smooth Muscle Cells using the IonFlux System

Introduction
Classical cell based screening assays employ cell lines in which the channel under study has been transfected to achieve elevated levels of channel expression. However, disparities exist between experimental results of screening assays using cells over expressing the target of interest as compared to cells found in vivo with endogenous levels of ion channel expression. This has raised concerns regarding the physiological relevance of the screening. In non-transfected cells, the assay targets are expressed in a more physiologically relevant environment, and the complex interplay of the endogenously expressed ion channels, secondary messengers and other cell signaling proteins can be better recapitulated than in the recombinant cell lines. Therefore, there is a growing interest in the use of non-transfected cells for drug discovery screening.

Patch clamp recording is the gold standard in studying ion channels. Automated patch clamp (APC) systems can achieve higher throughput than the conventional manual patch clamp and are less labor intensive, which allow greater capacity to conduct ion channel screening. To date, the most common APC assays use transfected cell lines. It is of great interest to explore the capability of the APC system recording of endogenous channels in non-transfected cells to achieve maximal native cell physiological relevance for compound screening studies.

In this report, we present recordings from endogenous $\text{GABA}_A$ channels in human airway smooth muscle cells using IonFlux automated patch clamp system. Dr. Charles Emala’s group (Columbia University) is the first to demonstrate the expression and function of endogenous ionotropic $\text{GABA}_A$ channels on human smooth muscle cells\(^1\). We show that IonFlux system can measure currents of these endogenous $\text{GABA}_A$ channels on the smooth muscle cells with a high success rate, and good quality whole cell, loose patch current.

Materials and Methods

Cells: Human bronchial smooth muscle cells were a kind gift from Dr. William Gerthof-fer (University of S. Alabama) and were immortalized by the stable expression of human telomerase reverse transcriptase (hTERT)\(^2\). Expression of hTERT extends the life span of smooth muscle cells, but does not effect the endogenous level of expressed channels. Cells were grown to confluence in 15cm diameter collagen coated (BD Biosciences (354236)) rat tail collagen type 1, 5 μg/cm\(^2\)) plastic dishes. Cells were grown in M199 media containing 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) in a humidified atmosphere of 5% CO\(_2\)/95% air at 37°C.

Preparation of cells: In preparation for recording, media was aspirated from the dishes and collagenase type IV (Sigma C5138) (500 un/ml in 5ml basal M199 media) was added, and the dishes were incubated 15min at 37°C in a cell culture incubator. Cells were released from the dish by gentle trituration using a pipette and transferred to a 15ml conical tube for centrifugation (300xg, 3 min, room temperature). The cell pellet was resuspended in the desired external buffer solution and washed twice. Cell solution (3~5x10\(^5\)/ml) was then loaded to the inlet wells in a 96well IonFlux microfluidic plate.

Experimental Procedures: The IonFlux plate layout consists of units of 12 wells; 2 wells contain intracellular solution, 1 contains extracellular solution plus cells, 8 contain compounds of interest, and 1 well is used for waste collection. A 96 well plate contains 8 units, and can provide 16 recordings (384 well plates provide 64 recording channels). Cells are captured from suspension by applying suction to microscopic channels in ensemble of recording arrays. Once the array is fully occupied by 20 individual cells, the applied suction breaks the cell membranes of captured cells, establishing whole cell voltage clamp.

Figure 1. The IonFlux system utilizes a “plate reader” format to simplify workflow and increase throughput. The instrument operates on microfluidic channels with fluidics exchange rate on 100ms timescale and the simultaneous recording. 20 cells per ensemble under voltage clamp are exposed to a compound within a short time scale in parallel across a 96 or 384 well plate. Throughputs of 10,000 data points per day can be achieved. It integrates a temperature control module, which enables the users to perform temperature dependent electrophysiology assay screening.

Figure 2. A: Endogenous GABA channel recording using the IonFlux system. Human airway smooth muscle cells were exposed to GABA with increasing concentrations from 10μM to 1000μM. B: The average seal from the smooth muscle cells on IonFlux was ~100Mohm, as compared with ~500 Mohm seal from fresh hGABA recombinant HEK cells , and was similar to the frozen cells. Success rate of the assay is defined as detectable current (>500pA). The success rate for smooth muscle cells was ~75%.

Abbreviations: APC = automated patch clamp, hGABA = human GABA, hTERT = human telomerase reverse transcriptase, HEK = human embryonic kidney cell
Recording of Endogenous Ion Channel Expression on IonFlux

For compound applications, pressure was applied to the appropriate compound wells, introducing the compound into the extracellular solution rapidly flowing over the cells. For recording of GABA currents, cells were clamped at a holding potential of −80mV. Each GABA application lasted for 3 seconds, followed by a 30 to 60 second wash to allow the channels to recover. Data analysis and graphical presentation were performed using a combination of IonFlux software, Microsoft Excel, and Origin Lab. All data are shown with arithmetic mean ± standard error of mean (SEM).

Results:
Figure 2. shows a GABA dose dependent response in human airway smooth muscle cells (A), as well as the average seal resistance and success rates for these smooth muscle cells (B). The inward Cl⁻ current from endogenous GABA₆ channels recorded on IonFlux showed clear dose dependency. This 20-cell ensemble of smooth muscle cells showed ~1.5nA current at the highest GABA concentration. The seal resistance from the smooth muscle cells is lower than the fresh hGABA₆(α1/β3/γ2) recombinant HEK cells (Millipore, Cat#: CYL3053), but is similar to the frozen recombinant HEK cells. Figure 3 showed a screen capture of Cl⁻ currents from the GABA₆ channels activation in smooth muscle cells across a whole plate (A). 12 out of 16 traces showed detectable currents (>500pA), or a success rate of ~75%.

Discussion

Traditional automated electrophysiology assays used for compound profiling rely on transfected cell lines that overly express the ion channel protein of interest. In order to increase the physiological relevance of such assays it is desirable to use tissue-derived cells that present endogenous expression of ion channel targets and contain other important features of the in-vivo environment such as secondary messengers and other cell signaling proteins. In this report, we demonstrate the feasibility of studying endogenous GABA₆ channel in human bronchial smooth muscle cells. The heterogeneity of cells resulted in a low success rate using manual patch clamp. The IonFlux platform records from 20-cell ensembles and features continuous compound perfusion; this approach had a success rate of 75% in terms of recording GABA₆ currents with good signal to noise ratios from immortalized primary cells. The data generated is in agreement with previous manual patch clamp recordings, suggesting that this approach is well suited to the interrogation of cultured primary cells. Recent work in Dr. Emala’s lab showed similar results using primary cells harvested from human airway smooth muscle; this demonstrated the capability of IonFlux to measure endogenous channels in ex vivo primary cells.

References

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