Comparison of cell expression formats for the characterization of the GABA<sub>A</sub> channels using IonFlux system


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Abstract

Ensemble recording and microfluidic perfusion are recently introduced techniques aimed at realizing the laborious novice and low recording success rates of manual patch clamp. Here, we present assay characteristics for these features integrated into one automated electrophysiology platform as applied to the study of GABA<sub>A</sub> channels. A variety of cell types and methods of GABA<sub>A</sub> channel expression were successfully studied (defined as GABA<sub>A</sub> > 50%) including stably transfected HEK cells expressing α1-5β2γ2δ GABA<sub>A</sub> channels, frozen “ready to assay” (RTA/HEK) cells expressing α1-2β2γ2δ GABA<sub>A</sub> channels, transiently transfected HEK293 cells expressing α1-2β2γ2δ GABA<sub>A</sub> channels, and immortalized cell lines of human embryonic stem cells endogenously expressing GABA<sub>A</sub> channels. We obtained success rates above 95% for transiently or stably transfected HEK cells and frozen “ready to assay” HEK cells expressing GABA<sub>A</sub> channels. Current measurements were successfully studied in multiple cell types with multiple routes of channel expression in response to several classic GABA<sub>A</sub> channel agonists, antagonists and allosteric modulators.

Materials and Methods

Reagents a-Amino-5-butyric acid, bicuculline, picrotoxin, muscimol and desipramine were purchased from Sigma-Aldrich, and used without further purification. H<sub>2</sub>SO<sub>4</sub> was prepared by Dr. James Cook.

Cell culture and preparation of recombinant GABA<sub>A</sub>, HEK293 cells

Cells expressing Yoda2i-δ (Ipswich Preclinical<sup>®</sup>, Ipswich, γ<sub>2</sub>δ-HEK Recombinant Cell Line. Cells (CCL303) were cultured in 175 cm<sup>2</sup> flasks containing DMEM/F12 (1:1), 10% fetal bovine serum (FBS), 1% pen-strep (100 μg/ml penicillin and 100 μg/ml streptomycin), 8 μg/ml insulin, 1 μg/ml human transferrin, 0.1 μM sodium selenite, 0.3 μg/ml EGF, and 0.2 μg/ml insulin. The cells were kept at 37°C with 5% CO<sub>2</sub> and 95% humidity.

Cell preparation of frozen RTA recombinant GABA<sub>A</sub> HEK293 cells

Two RTA cell lines, one expressing H<sub>2</sub>SO<sub>4</sub> iGABA<sub>A</sub> (delta 2) (Ipswich 17253576)(iGABA<sub>A</sub>) and the other one expressing H<sub>2</sub>SO<sub>4</sub> iGABA<sub>A</sub> (delta 2) (Ipswich 17253576)(iGABA<sub>A</sub>) were used for this study. For experiments, cells were grown in liquid nitrogen prior to use. For the assay, cells were thawed by immediately removing the vials in a 37°C water bath. As soon as the ice was thawed, 1 ml of pre-warmed 10% FBS serum-free media was added to the vials. The cells were washed 2 times in warm serum-free media and were incubated for 30 minutes at 37°C, washed and resuspended in extracellular solution at a density of 5-10<sup>6</sup> cells ml<sup>-1</sup> before the electrophysiology experiment.

Cell culture and preparation of immobilized human airway smooth muscle cells

Human bronchial smooth muscle cells were kindly gifted Dr. William Gerthoffer (University of S. Alabama) and immortalized by the stable expression of human tumor necrosis factor receptor superfamily (hTNFRSF) expressing HEK cells. hTNFRSF expression on HEK cells extends the life span of mature muscle cells, but does not affect the endogenous level of expressed receptors. Cells were originally transfected by human bronchial smooth muscle cell immortalization protocols. Cells were grown to confluence in 15 cm diameter collagen coated flat polystyrene Petri dish using 1:10<sup>6</sup> cells ml<sup>-1</sup>. Upon reaching 90% confluence, cells were grown in F12/F12 media containing 10% fetal bovine serum, 0.9 mM sodium selenite, 1 mM sodium pyruvate, 150 μM uridine, and 150 μM pyruvate. For 24 h, the media was exchanged BSA-free media. The mixture consisted of 15 ml of serum-free MEMs media, 5 μg of each of the receptor subunit DNA (iGABA<sub>A</sub>, iGABA<sub>B</sub>, iGABA<sub>C</sub>, iGABA<sub>D</sub>). After 24 hours, without changing media, the cells were washed 3 times with 10 ml of Ca<sup>2+</sup>-free media (iGABA<sub>A</sub> and iGABA<sub>B</sub> free media). Cells were then washed and resuspended in extracellular solution at a density of 5-10<sup>6</sup> cells ml<sup>-1</sup> before assay.

Conclusions

The GABA<sub>A</sub> assay is very well validated on the IonFlux<sup>™</sup> automated patch clamp system, in various expression formats/cell line combinations, frozen ready to assay recombinant cells, transiently transfected GABA<sub>A</sub> channels and immortalized human airway smooth muscle cells with endogenous GABA<sub>A</sub> channels.

Ensemble recording coupled with continuous fluid perfusion yields high success rates for the study of GABA<sub>A</sub> receptors either in transfected HEK293 cells or endogenously expressed in smooth muscle cells.

The IonFlux instrument with a small footprint, easy to use features, and robust assay performance makes the channel research accessible to a broader spectrum of researchers with no manual patch clamp experience, and facilitates small molecule screening in academic settings.
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