Characterization of hGABA<sub>A</sub>α<sub>1</sub>β<sub>3</sub>γ<sub>2</sub> on Nanion’s SyncroPatch96®

The electrophysiology team at Nanion Technologies GmbH, Munich, Germany. Cells were supplied by Millipore, USA.

Introduction

HEK-cells expressing GABA<sub>A</sub>-receptors were investigated with the SyncroPatch96 using a stacked application approach for rapid administration of compounds to the cells. The GABA receptor family is the most important class of inhibitory ion channels involved in synaptic transmission, and are selectively permeable to monovalent anions. They constitute an important therapeutic area for drugs affecting anxiety, sleep and muscle relaxation.

As with most ligand gated ion channels, GABA<sub>A</sub> exhibit receptor desensitization, which is a common phenomenon for ligand gated ion channels. Desensitization can be either exposure time dependent or concentration dependent, or both. Desensitization and recovery kinetics varies from milliseconds to tens of minutes, all depending on receptor type and subunit composition. For rapidly desensitizing ion channels, it is important that compound application is fast, so that the entire ion channel population is exposed to maximum concentration before entering the desensitized state.

Exposure time and application intervals are important factors affecting desensitization and recovery from desensitization, to minimize deleterious effects or receptor desensitization.

Results

In the experiments presented here, compounds were added to the cells with accurate timing ensuring a brief compound exposure time.

Figure 1 shows representative parameters of hGABA<sub>A</sub>α<sub>1</sub>β<sub>3</sub>γ<sub>2</sub> HEK cells as recorded on a NPC-96 planar patch clamp chip on the SyncroPatch96. 41.7% of the cells reached a seal resistance above 1 GΩ, 63.4% of cells reached a seal resistance above 500 MΩ. Seals remained stable throughout the experiment.
Methods

Cells
Millipore PrecisIoN™ hGABA_\textsubscript{A} \alpha1\beta2\gamma2 HEK cells. Cells were cultured and harvested according to Nanion’s standard cell culture protocol.

Cell Culture
Cells were cultured and harvested according to Nanion’s standard cell culture protocol.

Electrophysiology
Whole cell patch clamp recordings were conducted according to Nanion’s standard procedure for the SyncroPatch®.

Fig. 2 Graphical user interface of the screening software used on the SyncroPatch96. Screenshot of depiction of raw data traces of hGABA_\textsubscript{A} \alpha1\beta2\gamma2 expressing cells as recorded on one NPC-96 multihole (4x) patch clamp chip. Ninety-six small color-coded pictures as seen in the upper left part display 96 recordings. One highlighted experiment is displayed below, 16 other selected experiments are displayed on the right. Highlighted graphs show raw data current traces of the sum of 4 cells which were exposed to 3 μM GABA consecutively 7 times, including intermittent wash steps.

Figure 2 shows a screenshot of the SyncroPatch96 software. A color-coded overview of all 96 wells gives the user a good impression of the success rate of the experiment. The user can choose whether to visualize raw traces or online analysis. An individual well can be highlighted to monitor the progression of the experiment. For control recordings, we applied the EC\textsubscript{50} concentration (3 μM) seven times. The pharmacological experiment started with 3 initial control applications to evaluate current amplitude stability and a subsequent 3 point dose response curve followed by a washout.

Importantly, the ability to perform cumulative concentration response curves on single cells drastically reduces the consumable cost per data point. The SyncroPatch96 is a high throughput and highly reliable automated patch clamp device. User-friendly software, excellent success rates, multiple additions of compound to each cell and easy analysis result in high quality, reliable data at an increased throughput with an economical cost per data point.