Application Note

Channel: hGABA$_A$ α5β3γ2
Cells: HEK293
Tools: SyncroPatch96®

Characterization of hGABA$_A$ α5β3γ2 on Nanion's SyncroPatch96®

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Cells were supplied by Millipore, USA.

Introduction

HEK-cells expressing GABA$_A$-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$_A$ 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 deletrious 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.

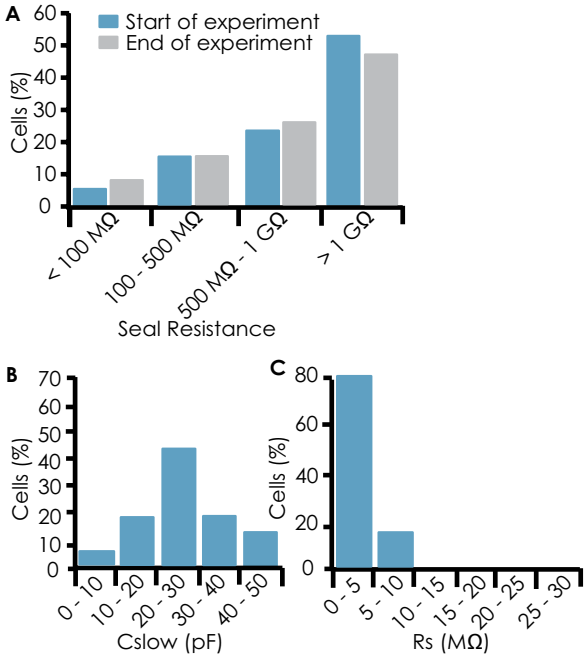

Fig.1 Statistic of hGABA$_A$ α5β3γ2 cells recorded on one NPC-96 patch clamp chip. C_{slow} = 26.2 ±1.8 (n=32), R_s = 4.1 ± 0.2 (n=32). 53 % of the cells on one NPC-96 chip (total n=96) had seal resistance > 1 Giga Ohm at the beginning, 47 % at the end of experiment.

Figure 1 shows representative parameters of hGABA$_A$ α5β3γ2 HEK cells as recorded on a NPC-96 planar patch clamp chip on the SyncroPatch96. 53 % of the cells reached a seal resistance above 1 GΩ, 76 % of cells reached a seal resistance above 500 MΩ. Seals remained stable throughout the experiment.

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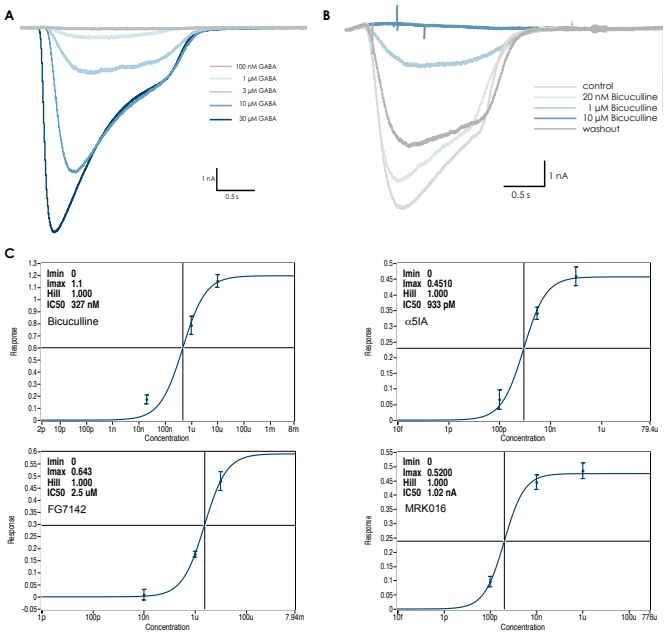

Fig. 2 Pharmacology on GABA$_A$ α5β3γ2 as recorded on the SyncroPatch96. Raw data traces of one exemplary cell using increasing GABA concentrations (**A**) or increasing Bicuculline concentrations and a subsequent washout (**B**). Cells were held at a constant holding potential of -70 mV and GABA was applied for approximately 2 s. After 3 control applications of 5 µM GABA, increasing concentrations of inhibitors were applied. Cells were pre-incubated for approx. 3 min in each concentration before co-application with 5 µM GABA. **C** Mean concentration response curves for Bicuculline, IC_{50} = 327 nM (n = 14); for α5IA IC_{50} = 933 pM (n = 11), maximum block was 45% at 100 nM; for FG7142 IC_{50}= 2.5 µM (n = 9), maximum block was 64.3% at 10 µM; for MRK016 maximum current inhibition was 52% at 1 uM, IC_{50}= 1.02 nM (n = 15).

Pharmacological experiment were performed using the inhibitors Bicuculline, α5IA, FG7142 and MRK016 (Fig. 2). Control applications for current amplitude stabilization were performed, followed by the application of 3 increasing inhibitor concentrations and a subsequent washout.

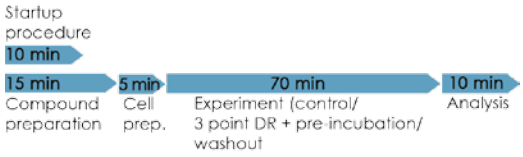

Fig. 3 Timeline of one run on the SyncroPatch96. The completion of an experiment on the SyncroPatch96 patch clamp chip (96 wells) for multiple 3-point concentration response curves plus control and washout at the end of the experiment on GABA$_A$ receptors took approx. 70 min.

Figure 3 offers a visual representation of an experiment with hGABA$_A$α5β3γ2 cells on the SyncroPatch96, resulting in vehicle control measurements (data not shown) and the dose response curves as shown in Fig. 2. From startup of the system, execution of the patch clamp experiment and analysis of the data it takes approx. 70 min. 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 for recording ligand-gated channels like GABA$_A$ receptors. 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.

Methods

Cells

Millipore PrecisION™ hGABA$_A$ α5β3γ2 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®.

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