Application Note

Characterization of hGABA$_A$ $\alpha_5\beta_3\gamma_2$ on Nanion’s SyncroPatch96®

The electrophysiology team at Nanion Technologies GmbH, Munich, Germany. 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 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.

Fig. 1 Statistic of hGABA$_A$ $\alpha_5\beta_3\gamma_2$ cells recorded on one NPC-96 patch clamp chip. $C_{slow} = 26.2 \pm 1.8$ (n=32), $R_s = 4.1 \pm 0.2$ (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$_A$ $\alpha_5\beta_3\gamma_2$ 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.

Download more Application Notes from www.nanion.de
Application Note

Fig. 2 Pharmacology on GABA$_{\text{a}5\beta3\gamma2}$ 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 μM; 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.

Methods

Cells
Millipore PrecisIO® hGABA$_{\alpha5\beta3\gamma2}$ 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®.