Ligand-activation of GABA<sub>A</sub> receptors on the automated patch clamp platforms QPatch and Qube 384 using conventional electrophysiology and optopharmacology


Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS) and the binding of GABA to ionotropic GABA receptors (GABA<sub>A</sub>) is a crucial process in the healthy brain. An imbalance of GABA secretion or the malfunction of the receptor is associated with multiple disease areas like anxiety disorders, seizures and schizophrenia. Pharmacological manipulation of the receptor has therefore a large therapeutic potential, which is underscored by the amount of available treatment possibilities and the ongoing search for alternatives thereof [1].

GABA<sub>A</sub> receptors are ligand gated ion-channels that consist of 5 membrane spanning subunits [2] and are permeable to Cl<sup>-</sup> ions. So far, 16 different subunits have been identified in humans (\(\alpha_1 - \alpha_6 \), \(\beta_1 - \beta_3 \), \(\gamma_1 - \gamma_6 \), \(\delta \), \(\epsilon \), \(\gamma \), \(\gamma \)) and their combination within the GABA<sub>A</sub> receptor leads to different pharmacological responses. Here, we show pharmacological modulation of the GABA<sub>A</sub> receptor using our high-throughput automated patch clamp (APC) systems QPatch and Qube 384. Our study includes a characterization of the heterogeneous GABA<sub>A</sub> receptor population of cultured primary hippocampal astrocytes and an evaluation of the GABA<sub>A</sub> receptor population in primary hippocampal astrocytes and hence the raw data rather than the average is plotted in the figure. The EC<sub>50</sub> value at 30 µM GABA: 3.3 µM.

Materials and methods

Stably expressing HEK cells

GABA<sub>A</sub>-deficient HEK293 cells were kindly provided by Charles River Laboratories and cultured according to the supplier’s description. All experiments were carried out at ambient temperature using QPatch or Qube multihole consumables and physiological solutions.

Compound addition and incubation

All agents were evaluated in the presence of GABA in the concentration indicated. For QPatch compound application, the antagonist was applied prior to a 3-second application of GABA + antagonist. On the Qube, the GABA application duration was 0.8 seconds, delivered by liquid loading.

Optopharmacology

RuBi-GABA from ToWy was applied and uncaged by a 475 nm light exposure.

Solutions

The extracellular solution contained (in mM): 145 NaCl, 10 HEPES, 4 KCl, 2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 1 MgCl<sub>2</sub>Oxide, pH 7.4; the intracellular solution 90 KCl, 50 KF, 110 EGTA, 10 Hepes, 4 MgATP, 1 MgCl<sub>2</sub>, 300 mMgCl, pH 7.3.

Data Analysis

Analysis was performed using the Sophion Analyzer, Origin 7.5 (OriginLab Corporation) and GraphPad Prism 7.03 (GraphPad Software Inc.).

Methods

1. Pharmacology of GABA<sub>A</sub>(α5β3δ2)-HEK293 cells

Fig. 1: Concentration-response relationship of GABA on hippocampal astrocytes. A: Typical responses to the 0.8 sec application of GABA in increasing concentrations. B: The response was normalized to the average at the highest GABA concentration. Error bars: ±SD. B: Normalized response vs concentration (n = 32). The response was plotted to show the pharmacology of a physiologic GABA response. The EC<sub>50</sub> of picrotoxin is consistent with what has been found for most GABA<sub>A</sub> receptors in neurones.

2. Pharmacology of primary hippocampal astrocytes (QPatch)

Fig. 2: Concentration-response relationship of the GABA receptor in hippocampal astrocytes. A: The EC<sub>50</sub> value at 30 µM GABA: 3.3 µM.

Fig. 3: Concentration-response relationship of bicuculline (200 nM) on the GABA receptor in hippocampal astrocytes. The EC<sub>50</sub> value at 30 µM GABA: 8.8 µM.

3. Optopharmacology of GABA<sub>A</sub>(α5β3δ2)-HEK293 cells (Qube)

Fig. 4: RuBi-GABA application response. A: Concentration-Dependent Response of Primary Mesencephalic Neuronal-Glia to RuBi-GABA. The EC<sub>50</sub> value at 30 µM GABA: 11.9 µM. 100% open channels at the 20 nM RuBi-GABA level. B: Normalized response vs concentration (n = 32). The response was plotted to show the pharmacology of a physiologic GABA response. The EC<sub>50</sub> of RuBi-GABA of a 3-fold dilution series. A Hill equation was fitted to the data and the calculated EC<sub>50</sub> was 11.9 µM (n = 13). Displayed are average values ± SE.

References