SOPHION BIOSCIENCE

Application Report

Ligand gated ion channels: $GABA_A$ receptor pharmacology on QPatch

Thorough compound evaluation in a $GABA_A(\alpha_5\beta_3\gamma_2)$ cell line on QPatch and on the GABA response of primary hippocampal rat astrocytes

Summary

Studies on $GABA_A$ ion channels using the automated patch clamp platform QPatch with the focus on:

- Effects of agonists, antagonists, and modulators
- Concentration-response relationships
- EC_{50} and IC_{50} determination
- Characterizing both the pharmacology of a specific isolated $GABA_A$ subtype and the physiological GABA response of cultured rat astrocytes

Introduction

The major inhibitory neurotransmitter of the central nervous system is γ-aminobutyric acid (GABA) and GABA is exerting its effect by binding to GABA receptors. The central role of GABA in the nervous system is underscored by the devastating consequences of pathophysiological changes in GABA signaling. Conversely, manipulation of GABA receptors can offer relief of a large group of neurological and psychiatric disorders. Pharmacological manipulation of $GABA_A$ has a large potential and ligands increasing the current will typically have anxiolytic, anticonvulsant, amnesic, sedative, hypnotic, euphoriant, and muscle relaxant effects[1–4].

$GABA_A$ receptors are ligand gated ion-channels, permeable to Cl^- ions, and are consisting of 5 membrane spanning subunits[5,6]. 16 different subunits are identified in humans (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, θ, π, ρ_{1-3}) and a physiological GABA response is hence composed by a heterogenous population of GABA receptors with significant different pharmacology[7]. Here we demonstrate pharmacological GABA receptor evaluation in both a stable transfected cell line containing only $\alpha_5\beta_3\gamma_2$ receptors and a primary cell culture of rat hippocampal astrocytes with a diverse GABA receptor population.

Results and discussion

The following compounds were evaluated in the $GABA_A(\alpha_5\beta_3\gamma_2)$ cell line:

- GABA (agonist)
- Bicuculline (competitive antagonist)
- Picrotoxin (non-competitive antagonist)
- Diazepam (positive allosteric modulator)
- GABA, picrotoxin and diazepam were also evaluated in rat hippocampal astrocytes

Compound evaluation in $GABA_A(\alpha_5\beta_3\gamma_2)$-HEK293 cells

GABA

The concentration-response relationship of GABA on the $GABA_A(\alpha_5\beta_3\gamma_2)$ receptor was evaluated on QPatch by exposing the cells to a 3 second application of GABA in increasing concentrations (figure 1).

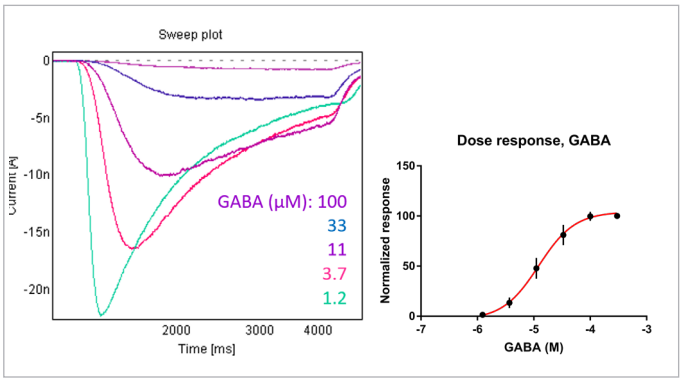

Fig. 1: GABA application experiments (left) and concentration-response relationship, n = 32 (right). Error bars: ± SD.

On average, 100 µM GABA elicited a 1.98 nA (± 0.64) response (peak current) per cell, the EC_{50} value was found to be 12.2 µM ($CI_{95\%}$: 11.1 to 13.4 µM) and the Hill slope to be 1.4 (±0.1).

Bicuculline

The concentration-response relationship of bicuculline was investigated and as bicuculline is a competitive antagonist, the response is both GABA and bicuculline concentration dependent. Hence, the cells were exposed to a 3 second application of 30 µM GABA in combination with an increasing concentration of bicuculine (figure 2). Prior to the GABA exposure, the cells were preincubated with the test concentration of bicuculline. The IC_{50} value at 30 µM GABA was found to be 3.3 µM ($CI_{95\%}$: 3.2 to 3.5 µM) and the Hill slope to be -1.8 (±0.06).

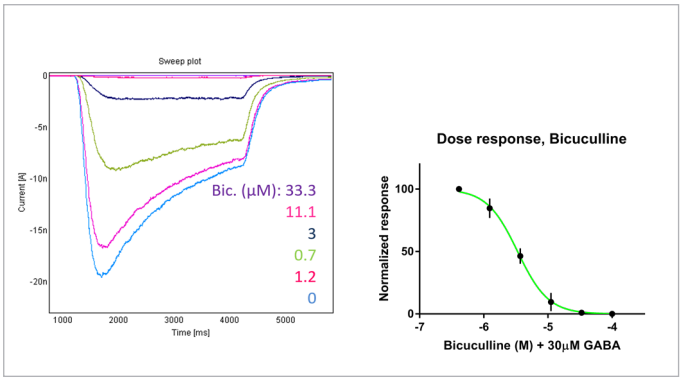

Fig. 2: Left: Bicuculline concentration-response experiment with increasing concentrations of bicuculline in the presence of 30 µM GABA. Right: dose-response curve, n = 20. Error bars: ± SD.

Picrotoxin

The non-competitive antagonist (pore blocker), picrotoxin was evaluated in the presence of 30 µM GABA. For each concentration, picrotoxin was washed in prior to a 3 second application of GABA. The IC_{50} value was found to be 0.8 µM ($CI_{95\%}$: 0.5 to 1.2 µM, figure 2) and the Hill slope to be -0.5 (±0.05).

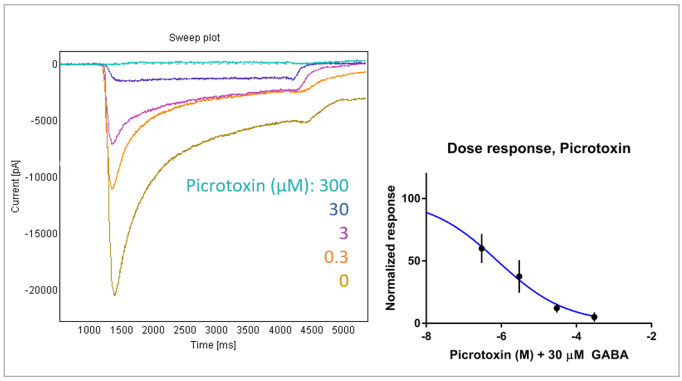

Fig. 3: Left: Picrotoxin concentration-response experiment with increasing concentrations of Picrotoxin in the presence of 30 µM GABA. Right: dose-response curve. Error bars: ± SD.

Diazepam

Diazepam is a classical benzodiazepine and hence a positive allosteric modulator of the $GABA_A$ receptor, potentiating the response to GABA. It exhibits GABA subunit selectivity, only potentiating receptors containing a α_1, α_2-, α_3- or α_5-subunit[7]. $GABA_A$ receptors only composed of α- and β-subunits (lacking γ) are insensitive to diazepam and the compound can therefore be used as an expression control. In the experiment, 4 µM GABA was applied either alone or in combination with 100 nM diazepam.

Diazepam did indeed potentiate the GABA response (figure 4), confirming the expression of all 3 subunits and the presence of correctly assembled $GABA_A$ receptors.

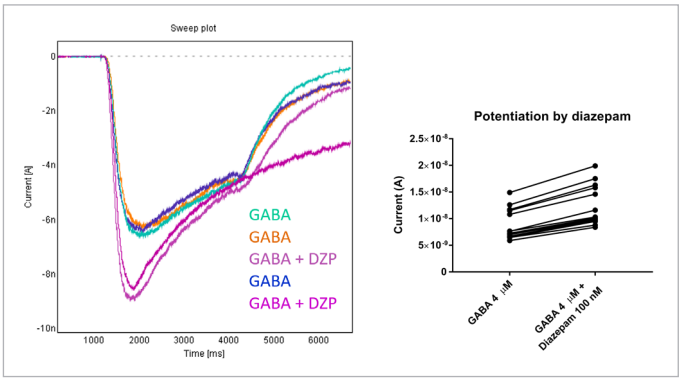

Fig. 4: Diazepam (100 nM) potentiated the GABA response (4 µM) in a reversible and reproducible manner (141% ± 16%, n = 17, p< 0.0001, paired students t-test). Left: representative trace from site. Note that there was no wash out after the last drug application, hence the different shape of the trace. Right: Plot showing the individual increase in GABA mediated current. Paired recordings are connected, showing how diazepam increased the current in all the experiments.

Compound evaluation in primary hippocampal astrocytes

In the body, the cellular GABA response is conducted by a population of GABA receptors with different subunit composition and different pharmacology, and the response will be a population response. To evaluate the pharmacology of a physiologic GABA response, we employ primary cell cultures of rat hippocampal astrocytes. The concentration-response relationship of GABA was evaluated by exposing the cells to a 3 second application of GABA in 8 increasing concentrations (figure 5).

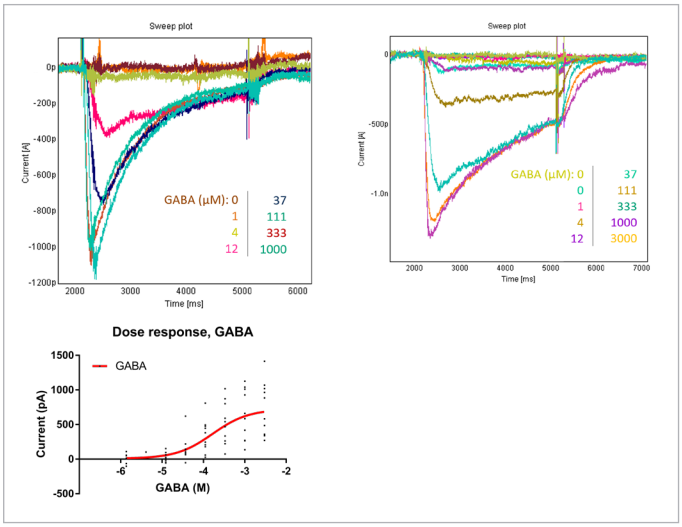

Fig. 5: Left: GABA responses to increasing GABA concentrations in two experiments and dose-response relationship. Right: dose-response curve, n = 12 (right).

On average, 3 mM GABA elicited a 70.2 pA current (±38.3 pA) per cell. There was a significant biological variation in the GABA response amongst the astrocytes and hence the raw data is plotted in figure 5. The EC_{50} value was found to be 161 µM ($CI_{95\%}$: 91.2 to 287 µM) and the Hill slope to be 0.9 (±0.5).

As expected, the size of this endogenous GABA current is only a fraction of the current found in the transfected HEK 293 cells (1.98 nA ± 0.64), where the GABA receptor is overexpressed, and the traces hence appears noisier, caused by the decreased signal to noise ratio.

Picrotoxin

Picrotoxin was evaluated in the presence of 1mM GABA. For each concentration, picrotoxin was washed in prior to a 3 second application of GABA. The IC_{50} value was found to be 2.2 µM ($CI_{95\%}$: 0.8 to 4.0 µM) and the Hill slope to be -0.6 (±0.3).

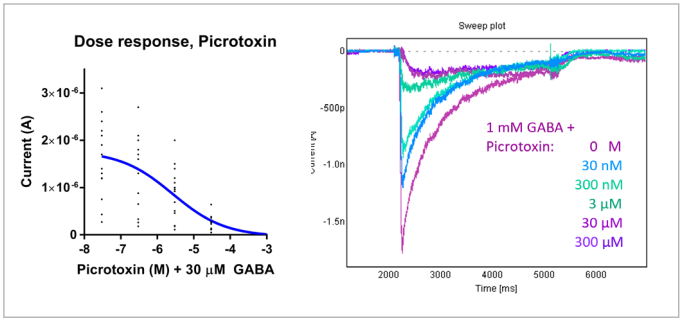

Fig. 6: Left: Picrotoxin concentration-response experiment with increasing concentrations of picrotoxin in the presence of 1 mM GABA. Experimental protocol: GABA 10 µM, GABA 10 µM, GABA 1 mM + picrotoxin 30 nM, 300 nM, 30 µM and 300 µM. Right: dose-response curve, n = 13.

The found IC_{50} for value picrotoxin is consistent with what generally is found for most $GABA_A$ receptors and the fact that the current is picrotoxin sensitive confirms the current being true $GABA_A$ current.

Methods

$GABA_A(\alpha_5\beta_3\gamma_2)$/HEK 293

$GABA_A(\alpha_5\beta_3\gamma_2)$/HEK 293 cells were cultured according to the supplier's description. All experiments were carried out at ambient temperature using QPatch multi-hole consumables and patched using a standard whole cell protocol and physiological solutions.

Primary rat hippocampal astrocyte cultures

The hippocampi were isolated from 1-5 d-old rat pups and astroglia-enriched cultures were grown according to Liu et al., 2003[8]. Patch clamp: All experiments were carried out at ambient temperature using QPatch multi-hole consumables and physiological solutions.

Data Analysis

Analysis was performed using the Sophion Assay Software and GraphPad Prism 7.03 (GraphPad Software Inc.).

References:

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