SOPHION BIOSCIENCE

Application Report

Pharmacological evaluation of $GABA_A$ receptor subtypes on Qube 384

High-throughput screening and cumulative concentration-response relationship of a $GABA_A(\alpha_5\beta_3\gamma_2)$ cell line. Characterization of $GABA_A$ receptor subtypes

Summary

Studies of $GABA_A$ ion channels using the automated patch clamp platform Qube 384 with focus on:

- Short ligand exposure with repetitive stimulations with EC_{50} concentrations of GABA
- Effects of agonists, antagonists and modulators
- Cumulative and non-cumulative concentration-response relationships
- Characterizing the pharmacological properties of four cell lines expressing different $GABA_A$ subtypes

Introduction

γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter of the central nervous system and pathophysiological changes in GABA signalling is the leading cause in a large group of neurological and psychiatric disorders including epilepsy, schizophrenia and depression. Consequently, manipulation of the GABA signalling holds a great therapeutic potential[1–4].

Ionotropic GABA receptors consist of 5 membrane-spanning subunits[5,6], of which 19 different have been identified in humans ($\alpha_{1\text{-}6}$, $\beta_{1\text{-}3}$, $\gamma_{1\text{-}3}$, δ, ε, θ, π, $\rho_{1\text{-}3}$). Receptors in different cell types have different subunit composition and differ both in pharmacology and subcellular location[7]. Due to the heterogeneity of $GABA_A$ receptors, they can perform different inhibitory tasks: Positive modulation of $GABA_A$ receptors can be anticonvulsant, hypnotic, anaesthetic and anxiolytic, whereas negative modulation can enhance cognition but also be anxiogenic and proconvulsant[1–4].

Here we use four $GABA_A$/HEK293 cell lines and a range of tool compounds to demonstrate two different approaches to $GABA_A$ receptor evaluation on Qube. We show 1) a cumulative dose-response experiment with a $GABA_A(\alpha_5\beta_3\gamma_2)$ cell line and 2) a $GABA_A$ receptor subtype screen, using a single compound plate layout fitting various receptor subtypes. This compound plate enables evaluation of both non-cumulative concentration-response relationships of GABA and bicuculline (competitive antagonist) and the potentiation with diazepam (positive allosteric modulator), simultaneously.

Results and discussion

Short agonist exposure, $GABA_A(\alpha_5\beta_3\gamma_2)$

Desensitization due to prolonged or repeated agonist exposure poses a challenge when studying $GABA_A$ receptors in an in vitro setting. Therefore, the effect of GABA on the $GABA_A(\alpha_5\beta_3\gamma_2)$ receptor was evaluated on Qube, employing the stacked delivery feature, where both the GABA-containing solution and the washout solution are stacked in the pipette. In this way, the exposure time is reduced to less than one second.

With the stacked delivery feature, consecutive applications of GABA (12 µM) could be made without significant rundown (Figure 1A). 12 µM GABA, which is close to the EC_{50} value, elicited on average 19.6 nA (±5.7 nA, SD) current pr. site using a multihole (x10 patch holes) QChip (see Figure 1B for a QChip view).

The success rate was up to 88% per plate with the following criteria:

Resistance > 100 MΩ per cell

Capacitance > 5 pF per cell

Current amplitude at 12 µM GABA > 300 pA per cell

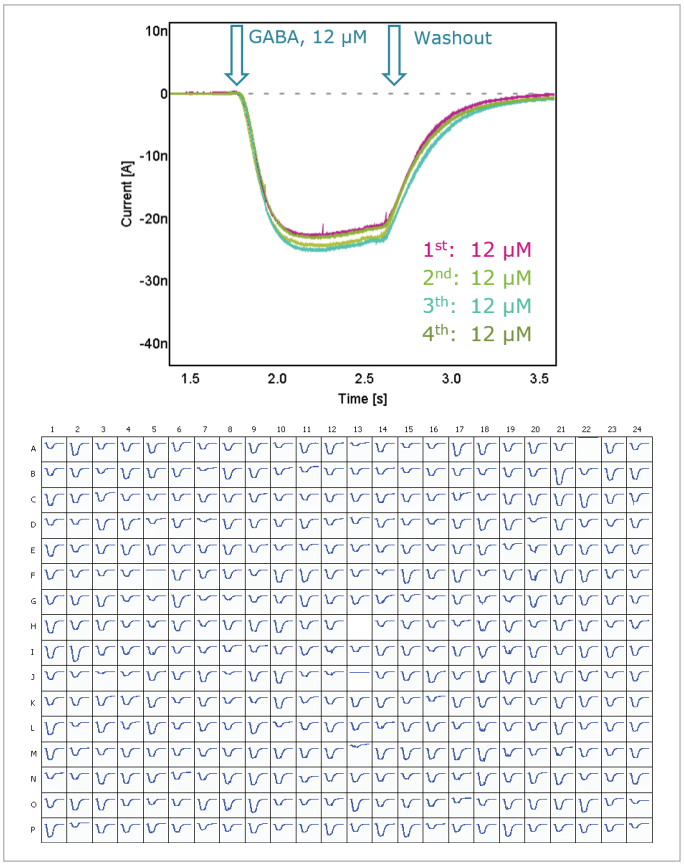

Fig. 1: Top: 4 consecutive applications of 12 µM GABA using the stacked delivery feature. Bottom: Plate view of a QChip, showing the 384 individual responses to 12 µM GABA.

Cumulative GABA concentration response

The concentration-response relationship of GABA on the $GABA_A(\alpha_5\beta_3\gamma_2)$ receptor was evaluated on Qube. GABA was applied in increasing concentrations (3-fold dilution from 400 µM, Figure 2). The EC_{50} value for the cumulative concentration response was found to be 10.9 µM (CI95%: 10.0 to 12.4 µM) and the Hill slope was 1.5 (±0.1, SD).

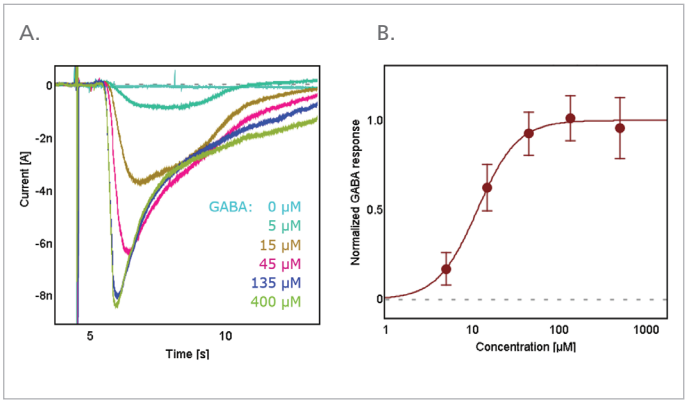

Fig. 2: Cumulative concentration-response relationship of GABA binding to the $GABA_A(\alpha_5\beta_3\gamma_2)$ receptor. A) Typically recorded currents in response to increasing concentrations of GABA. B) Peak current (normalized to highest value in experiment, average ± SD, n=192) as a function of GABA concentration for the whole QChip.

$GABA_A$ receptor subtype screen

The $GABA_A$ receptor subtypes differ both in pharmacology and physiological function. We designed a compound plate layout (Figure 3) for the screen, using one compound plate, four QChips and four cell lines to evaluate the pharmacological properties of four $GABA_A$ receptor types ($\alpha_5\beta_3\gamma_2$, $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_3\gamma_2$ and $\alpha_4\beta_3\delta$). The experiment results included non-cumulative GABA and bicuculline (competitive antagonist) concentration-response experiments. In addition, the potentiation of diazepam (positive allosteric modulator) was evaluated at different concentrations.

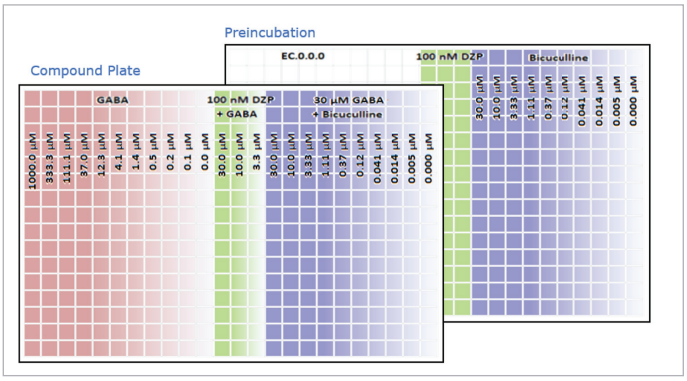

Fig. 3: Compound plate layout: 100 nM diazepam and varying concentrations of bicuculline were washed in (pre-incubation) prior to GABA application (compound plate).

The results of the screen are displayed in Figure 4-7 for the four different receptor types ($\alpha_5\beta_3\gamma_2$, $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_3\gamma_2$ and $\alpha_4\beta_3\delta$).

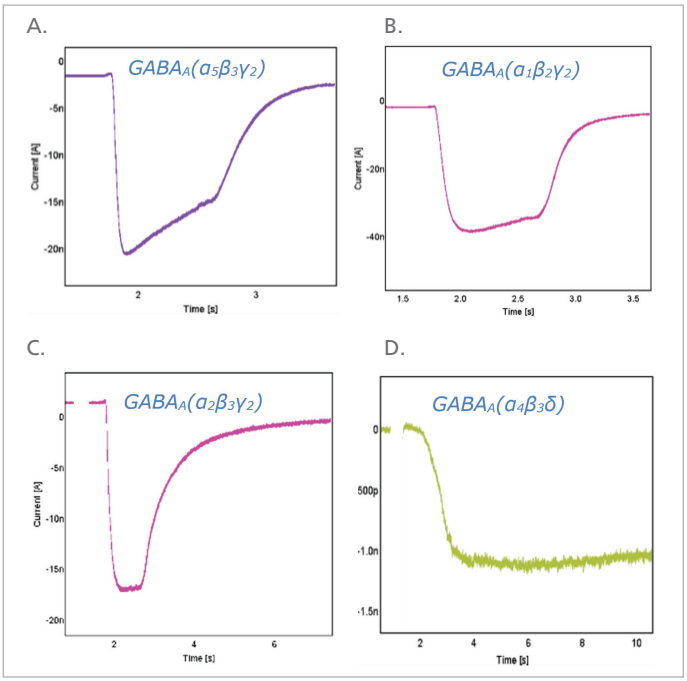

Fig. 4: $GABA_A$ receptor kinetics. Typical recordings from one multihole site (10 cells/site). The effect of GABA was evaluated employing the stacked delivery feature, with an exposure time of less than one second (with exception from $GABA_A$ ($\alpha_2\beta_3\delta$) where a regular and not stacked delivery feature was employed due to the slow kinetics of δ-subunit containing $GABA_A$ receptors).
A) $GABA_A$ ($\alpha_5\beta_3\gamma_2$): On average, 111 µM GABA elicited a 26.3 nA (± 8.5 nA) response (peak current). B) $GABA_A$ ($\alpha_1\beta_2\gamma_2$): On average, 111 µM GABA elicited a 31.6 nA (± 8.3) response (peak current). C) $GABA_A$ ($\alpha_2\beta_3\gamma_2$): On average, 1 mM GABA elicited a 9.6 nA (± 4.1) response (peak current). D) $GABA_A$ ($\alpha_4\beta_3\delta$): On average, 111 µM GABA elicited a 0.54 nA (± 0.17) response (peak current). Deviations are ± SD.

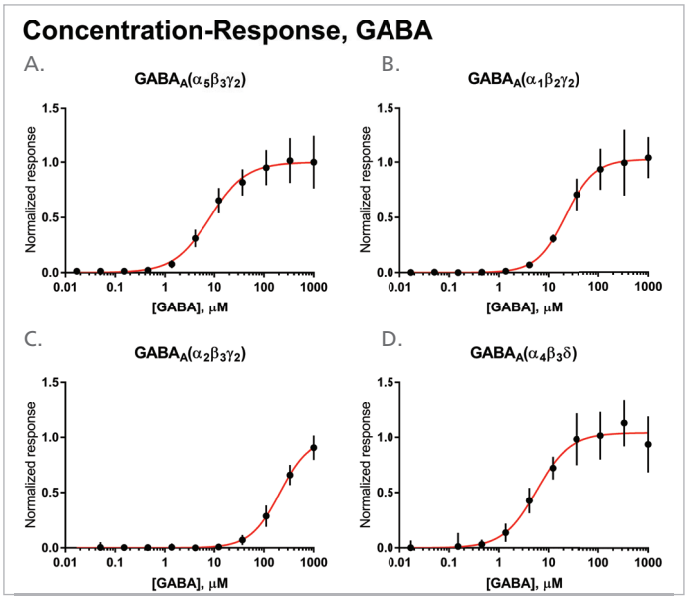

Fig. 5: GABA concentration-response of the four GABA$_A$ receptor subtypes.
A) For GABA($\alpha_5\beta_3\gamma_2$) the EC$_{50}$ value was 10.4 µM (CI95%: 8.5 to 13.7 µM) and the Hill slope was 1.2 (±0.07).
B) For GABA$_A$($\alpha_1\beta_2\gamma_2$) the EC$_{50}$ value was 22.1 µM (CI95%: 19.6 to 25.0 µM) and the Hill slope was 1.5 (± 0.1).
C) For GABA$_A$($\alpha_2\beta_3\gamma_2$) the EC$_{50}$ value was 0.21 mM (CI95%: 0.19 to 0.23 µM) and the Hill slope 1.45 (±0.08).
D) For GABA$_A$($\alpha_4\beta_3\delta$) the EC$_{50}$ value was 5.7 µM (CI95%: 4.5 to 7.3 µM) and the Hill slope was 1.2 (±0.2).
Error bars: ± SD.

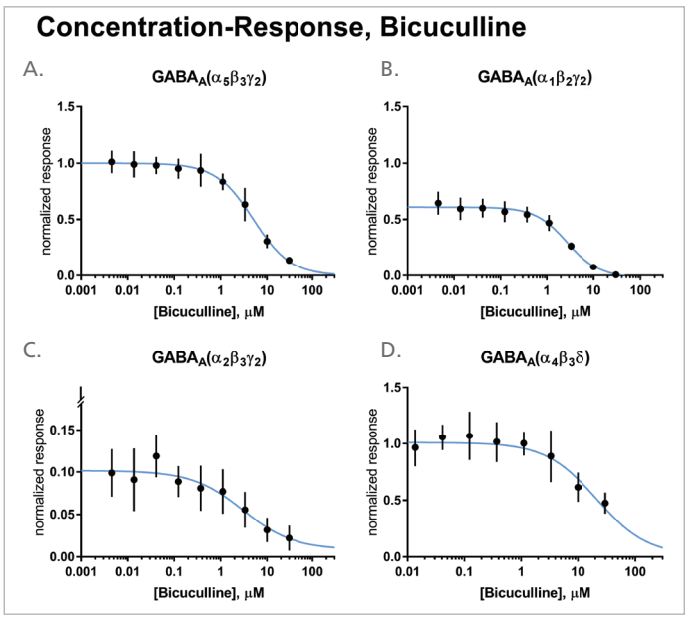

Fig. 6: Bicuculline concentration-response relationship of four GABA$_A$ receptor subtypes. Prior to the exposure to 30 µM GABA, the cells were preincubated with the test concentration of bicuculline. The response is normalized to the max GABA current.
A) For GABA$_A$($\alpha_5\beta_3\gamma_2$) the IC$_{50}$ value at 30 µM GABA was 5.1 µM (CI95%: 4.4 to 5.8 µM) and the Hill slope was -1.1 (±0.06).
B) For GABA$_A$($\alpha_1\beta_2\gamma_2$) the IC$_{50}$ value at 30 µM GABA was 2.7 µM (CI95%: 2.5 to 3.1 µM) and the Hill slope was -1.3 (±0.08).
C) For GABA$_A$($\alpha_2\beta_3\gamma_2$) the IC$_{50}$ value at 30 µM GABA was 3.8 µM (CI95%: 2.4 to 5.7 µM) and the Hill slope was -0.63 (± 0.08).
D) For GABAA($\alpha_4\beta_3\delta$) the IC50 value at 30 µM GABA was 16 µM (CI95%: 12 to 21 µM) and the Hill slope was -1.1 (±0.1).
Error bars: ± SD.

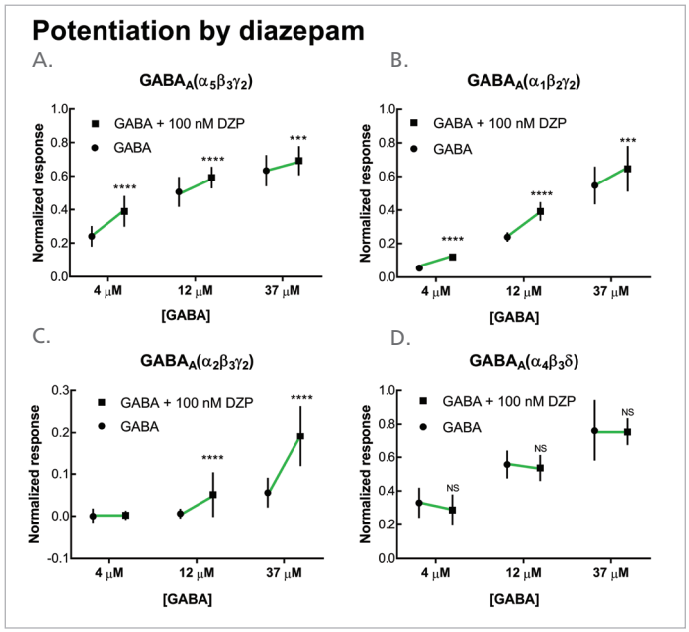

Fig. 7: Potentiation by diazepam. Diazepam is a benzodiazepine, binding to the benzodiazepine binding site which is located between the α and the γ subunit of the GABA$_A$ receptors. A diazepam effect can hence confirm a prober γ subunit expression.
A) Diazepam (100 nM) potentiated the GABA$_A$($\alpha_5\beta_3\gamma_2$) response: 4 µM: 162% ± 7%, p< 0.0001, 12 µM: 117% ± 3%, p< 0.0001 and 37 µM: 109% ± 3%, p< 0.05.
B) Diazepam (100 nM) potentiated the GABA$_A$($\alpha_1\beta_2\gamma_2$) response: 4 µM: 222% ± 7%, p< 0.0001, 12 µM: 164% ± 5%, p< 0.0001 and 37 µM: 116% ± 6%, p< 0.01.
C) Diazepam (100 nM) potentiated the GABA$_A$($\alpha_2\beta_3\gamma_2$) response. 4 µM GABA did however not induce a measurable response and the response to 12 µM GABA was only measurable in the presence of diazepam. The response to 37 µM GABA was increased by 100nM Diazepam: 341% ± 27%, p< 0.0001.
D) Diazepam (100 nM) did not potentiate the GABA response. A δ-subunit-containing GABA$_A$ receptor does not contain a benzodiazepine binding site, hence the lack of diazepam effect.
Error bars: ± SD.

Methods

The four HEK cell lines expressing human $GABA_A$ receptors were cultured according to the suppliers' description. ($\alpha_5\beta_3\gamma_2$)/HEK 293 was kindly supplied by Charles River Laboratories, Cleveland, OH, ($\alpha_5\beta_3\gamma_2$)/HEK 293 and ($\alpha_5\beta_3\gamma_2$)/HEK 293 was kindly supplied by SB Drug Discovery, Glasgow, UK and ($\alpha_5\beta_3\gamma_2$)/HEK 293 was kindly supplied by B'SYS, Witterswil, CH.

All experiments were carried out at ambient temperature using Qube 384 multi-hole consumables and patched using a standard whole-cell protocol.

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

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Author:

Kim Boddum , Application scientist

Sophion Bioscience A/S, Baltorpvej 154, 2750 Ballerup, Denmark
Phone: +45 4460 8800, E-mail: info@sophion.com

sophion.com