$GABA_A$ receptor pharmacology evaluted in overexpressing HEK cells and primary astrocytes on QPatch

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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].

GABAA receptors are ligand-gated ion channels, permeable to Cl^- ions, consisting of 5 membrane spanning subunits[5,6]. 16 different subunits are identified in humans ($\alpha_{1\text{-}6}$, $\beta_{1\text{-}3}$, $\gamma_{1\text{-}3}$, δ, ε, θ, π) and the cellular GABA response is hence composed by a population of GABA receptors with significant different pharmacology[7]. Here we demonstrate pharmacological GABA receptor evaluation in both a stably-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.

Methods

Stably expressing HEK cells

$GABA_A(\alpha_5\beta_3\gamma_2)$/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.

Antagonist application

All antagonist was evaluated in the presence of GABA. For each compound application, the antagonist was applied prior to a 3 second application of GABA + antagonist.

Data Analysis

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

Conclusions

In our study we demonstrated a pharmacological GABA receptor evaluation of both a single $GABA_A$ receptor clone ($\alpha_5\beta_3\gamma_2$), stably expressed in a HEK cell line, and in the heterogeneous GABA receptor population of cultured primary hippocampal astrocytes, using the automated patch clamp platform, QPatch.

References

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

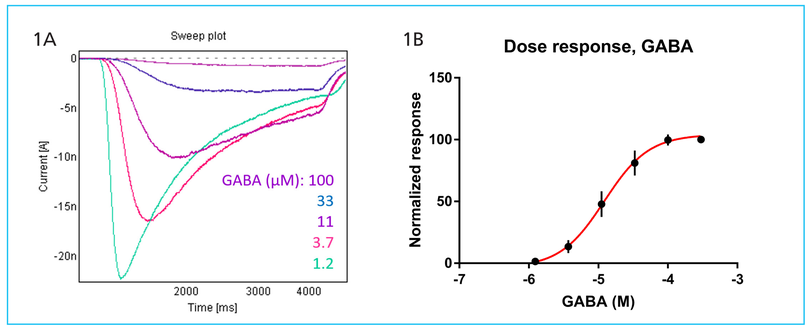

Fig. 1: **The concentration-response relationship of GABA on the GABAA($\alpha_5\beta_3\gamma_2$) receptor**
A: Typical responses to the 3 second application of GABA in increasing concentrations. B: Normalized response vs concentration (n = 32). Error bars: ± SD. EC_{50}: 12.2 µM ($CI_{95\%}$: 11.1 to 13.4 µM).

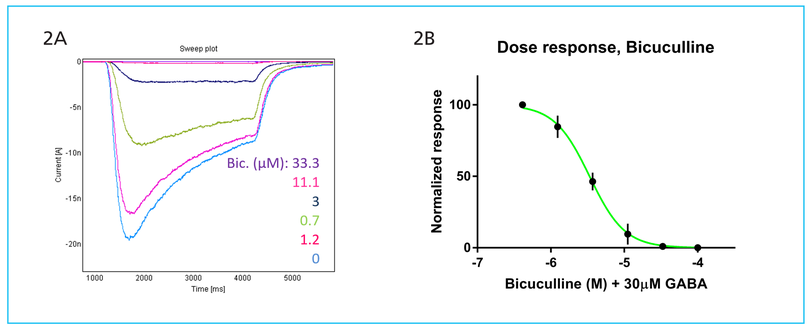

Fig. 2: **The concentration-response relationship of bicuculline**
A: Concentration-response relationship of the competitive antagonist bicuculline in the presence of 30 µM GABA. B: Plot of the normalized response vs concentration (n = 20). Error bars: ± SD. The IC_{50} value at 30 µM GABA: 3.3 µM ($CI_{95\%}$: 3.2 to 3.5 µM).

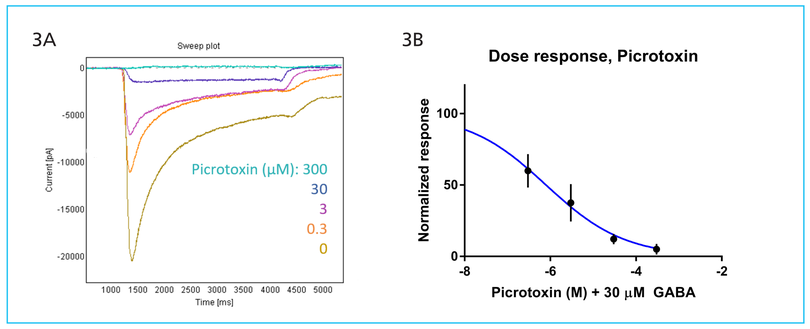

Fig. 3: **The concentration-response relationship of picrotoxin**
A: The pore blocker, picrotoxin, was evaluated in the presence of 30 µM GABA. B: Normalized response vs concentration (n = 20). Error bars: ± SD. IC_{50} value: 0.8 µM ($CI_{95\%}$: 0.5 to 1.2 µM)

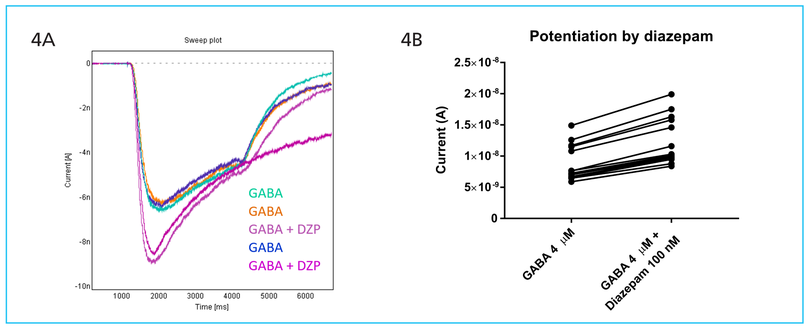

Fig. 4: **Potentiation of the $GABA_A(\alpha_5\beta_3\gamma_2)$ receptor by diazepam**
A: Typical traces of 4 µM GABA applied either alone or in combination with 100 nM diazepam. Note the attentional lag of wash out after the last drug application, hence the different shape of the trace. B: Individual increase in GABA mediated current. Diazepam did indeed potentiate the GABA response in a reversible and reproducible manner (141% ± 16%, n = 17, p< 0.0001, paired students t-test). Paired recordings are connected.

Compound evaluation in primary hippocampal astrocytes

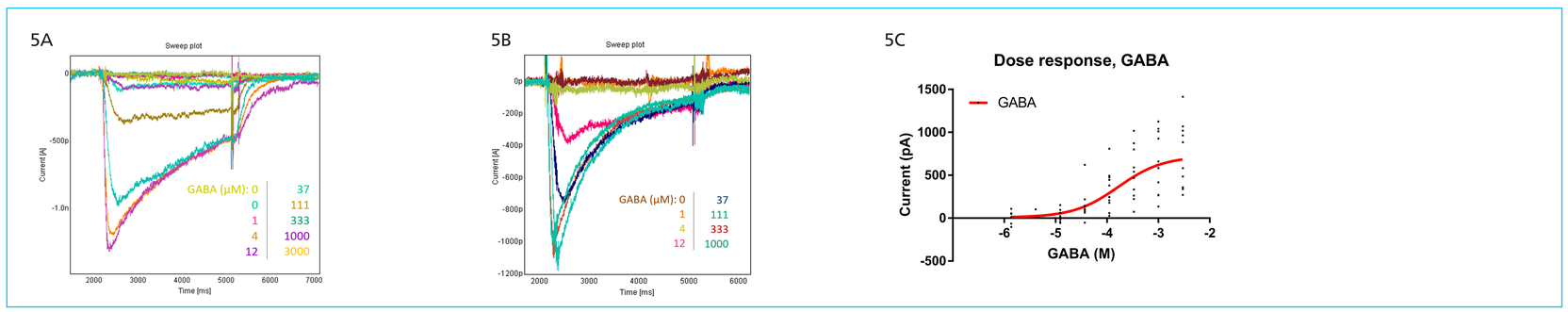

Fig. 5: **The concentration-response relationship of GABA on hippocampal astrocytes**
The cellular GABA response is conducted by a population of GABA receptors with different subunit composition and different pharmacology, and thus the response will be a population response. To evaluate the pharmacology of a physiologic GABA response, we employ primary cell cultures of rat hippocampal astrocytes. A & B: Typical recordings from astrocytes exposed to a 3 second application of GABA in 8 increasing concentrations. C: Concentration-response relationship of GABA on hippocampal astrocytes (n = 12). There was a significant biological variation in the GABA response amongst the astrocytes and hence the raw data rather than the average is plotted in the figure. The EC_{50} value was found to be 161 µM ($CI_{95\%}$: 91.2 to 287 µM). As expected, the size of this endogenous GABA current is only a fraction of the current found in the transfected HEK 293 cells, where the GABA receptor is overexpressed. The traces appear noisier, caused by the decreased signal to noise ratio.

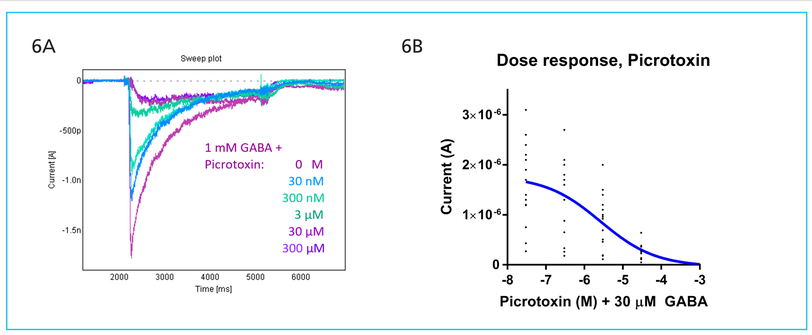

Fig. 6: **The concentration-response relationship of pwwicrotoxin on hippocampal astrocytes**
A: Typical traces recorded from an astrocyte exposed to 1 mM GABA and increasing concentrations of picrotoxin was evaluated. B: Plot of the current vs concentration (n = 13). IC_{50}: 2.2 µM ($CI_{95\%}$: 0.8 to 4.0 µM).
The found IC_{50} value for picrotoxin is consistent with what is found for most $GABA_A$ receptors and the fact that the current is picrotoxin sensitive confirms the current being true $GABA_A$ current.