Presented @ICMS2018

**GABA<sub>A</sub> receptor pharmacology evaluated in overexpressing HEK cells and primary astrocytes on QPatch**

Kim Boddum<sup>1</sup>, Kadia Raskova Rosholm<sup>1,2</sup>, Linda Blomster<sup>2</sup>, Hervé Lykke Olsen, Naja Møller Sørensen<sup>1</sup>, Göran Mattsson<sup>1</sup>

<sup>1</sup>Sophion Bioscience A/S, Ballerup, Denmark, 2 Saniona, Ballerup, Denmark, 1 Presenting author, E-mail: kn@ sophion.com

---

**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<sub>A</sub> has a large potential and ligands increasing the current will typically have anxiolytic, anticonvulstive, amnestic, sedative, hypnotic, euphoriant, and muscle relaxant effects.<sup>1-5</sup>

GABA<sub>A</sub> receptors are ligand-gated ion channels, permeable to Cl<sup>-</sup> ions, consisting of 5 membrane spanning subunits (α<sub>1-6</sub>, β<sub>1-3</sub>, γ<sub>1-6</sub>, δ, θ, Ϲ).<sup>6,7</sup> The cellular GABA response is response is composed by a population of GABA receptors with significant different pharmacology.<sup>8-10</sup> Here we demonstrate pharmacological GABA receptor evaluation in both a stably-transfected cell line containing only α<sub>5</sub>β<sub>2</sub>γ<sub>2</sub> receptors and a primary cell culture of rat hippocampal astrocytes with a diverse GABA receptor population.

**Methods**

Stably expressing HEK cells

GABA<sub>A</sub>(α<sub>5</sub>β<sub>2</sub>γ<sub>2</sub>)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 dissected from 1-5 day-old rat pups and astroglia-enriched cultures were grown according to Liu et al., 2003. 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 evaluation, 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<sub>A</sub> receptor clone (α<sub>5</sub>β<sub>2</sub>γ<sub>2</sub>) 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**


---

**Compound evaluation in GABA<sub>A</sub>(α<sub>5</sub>β<sub>2</sub>γ<sub>2</sub>)-HEK293 cells**

**Fig. 1:** The concentration-response relationship of GABA on the GABA<sub>A</sub>(α<sub>5</sub>β<sub>2</sub>γ<sub>2</sub>) receptor

A: Typical response to the 2 second application of GABA in increasing concentrations. B: Normalized response vs concentration (n = 32). Error bars: ± SD. IC<sub>50</sub>: 12.2 µM (CI<sub>95%</sub>: 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<sub>50</sub> value at 30 µM GABA: 3.1 µM (CI<sub>95%</sub>: 2.3 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 = 13). IC<sub>50</sub>: 0.8 µM (CI<sub>95%</sub>: 0.5 to 1.2 µM).

**Fig. 4:** Potentiation of the GABA<sub>A</sub>(α<sub>5</sub>β<sub>2</sub>γ<sub>2</sub>) 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 traces. 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 IC<sub>50</sub> value was found to be 161 µM (CI<sub>95%</sub>: 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.

---

**References**