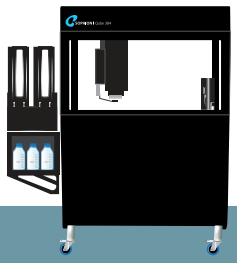
SOPHION BIOSCIENCE



Application Report

Investigating antipsychotic compound interactions with $GABA_A$ receptors in primary hippocampal neurons

Using high throughput automated patch clamp for exploratory compound screening reveals potential GABAergic effects of common antipsychotics

Summary

This study aimed to establish protocols for testing compound effects in primary hippocampal neurons, with a sub-focus on GABA-elicited effects. Dissociated primary mouse neurons are excellent modelling systems for neurobiological, biophysical, and pharmacological evaluations. The presence of a wide variety of ion channels and receptors ensures a physiologically relevant analysis of cell response and signalling. Patch clamp provides direct functional, temporal and spatial information of a cell's electrical and signalling properties. In addition, the Qube automated patch clamp (APC) platform enables a high throughput screening (HTS) by recording 384 cells simultaneously.

Several findings have led us to investigate further the relevance of the inhibiting actions of antipsychotic medications on GABAergic activity. Here we show data on:

- HEK cells expressing α_5-containing $GABA_A$ receptors and primary hippocampal neurons were used to screen a small library of antipsychotics.
- Isolated primary hippocampal neurons from mice were patched on the Qube 384 APC system. Using an optimized cell dissociation protocol to obtain healthy cell membranes for patch-clamp, we obtained a whole-cell success rate of up to 65%.
- The effect of 12 GABA receptor modulators on responses to sub-µM GABA concentrations (0.5 µM GABA) and found most inhibiting GABA currents, ranging from weak to full inhibition.
- The modulators showed similar results in $\alpha_5\beta_3\gamma_2$ $GABA_A$ receptors in HEK cells.

Introduction

α_5-containing $GABA_A$ receptors are mainly inhibitory, extrasynaptic receptors in the central nervous system and are preferentially expressed in the hippocampus (Marques *et al.*, 2021; Xu & Wong, 2018). These receptors have been implicated in the pathophysiology of schizophrenia and have been shown to play an important role in cognition, as well as learning and memory (Jacob, 2019). Similarly, dysfunction of the hippocampus has been associated with schizophrenia (Lieberman *et al.*, 2018; Lodge & Grace, 2011; Nakahara, Matsumoto, & van Erp, 2018).

Compounds targeting α_5-containing $GABA_A$ receptors (specifically negative allosteric modulators or NAMs) have shown promise in previous studies, as well as clinical trials, especially for the treatment of cognitive impairment in Down syndrome and schizophrenia (Atack *et al.*, 2006; Glykys, Mann, & Mody, 2008; Xu & Wong, 2018). Several pharmacological and genetic studies have shown that reducing α_5-containing $GABA_A$ receptor function can enhance cognitive abilities (Atack *et al.*, 2006; Collinson *et al.*, 2002; Zurek, Bridgwater, & Orser, 2012). Additionally, antipsychotics exert inhibitory effects on $GABA_A$ receptors, which are being considered for their physiological relevance to the antipsychotic action of these drugs (Bampali *et al.*, 2022; Lu *et al.*, 2023).

In this study, we aimed to examine the effects of antipsychotic compounds using Sophion's automated patch clamp systems. First, we created a small library of clinically relevant antipsychotics and assessed the functional activity of the compounds in HEK cells expressing the highly relevant $\alpha_5\beta_3\gamma_2$ $GABA_A$ receptors. We found that the compounds tested reduce GABA-induced currents, as previously predicted. Next, we explored the effects of the selected compounds in a more physiologically relevant system, namely primary hippocampal neurons, after we first electrophysiologically characterized them using Sophion's automated patch clamp systems. The results show varied levels of inhibition of GABA-elicited currents but are consistent with previous findings indicating that many antipsychotic compounds seem to suppress GABA currents (Bampali *et al.*, 2022).

Results and discussion

After the selection of a small library of antipsychotic compounds (numbered 2-12), the identity of which is not disclosed in this report, we tested them in HEK cells expressing $\alpha_5\beta_3\gamma_2$ $GABA_A$ receptors. After establishing GABA dose-response curves and the EC_{50} concentration for this receptor subtype, we proceeded to test all compounds at a concentration of 100 µM co-applied with the GABA EC_{50}. As shown in Figure 1C, all antipsychotic compounds inhibit GABA-induced currents with effects ranging from weaker (e.g. compounds 3, 9 and 11) to almost full inhibition (compounds 4, 6, 8 and 10).

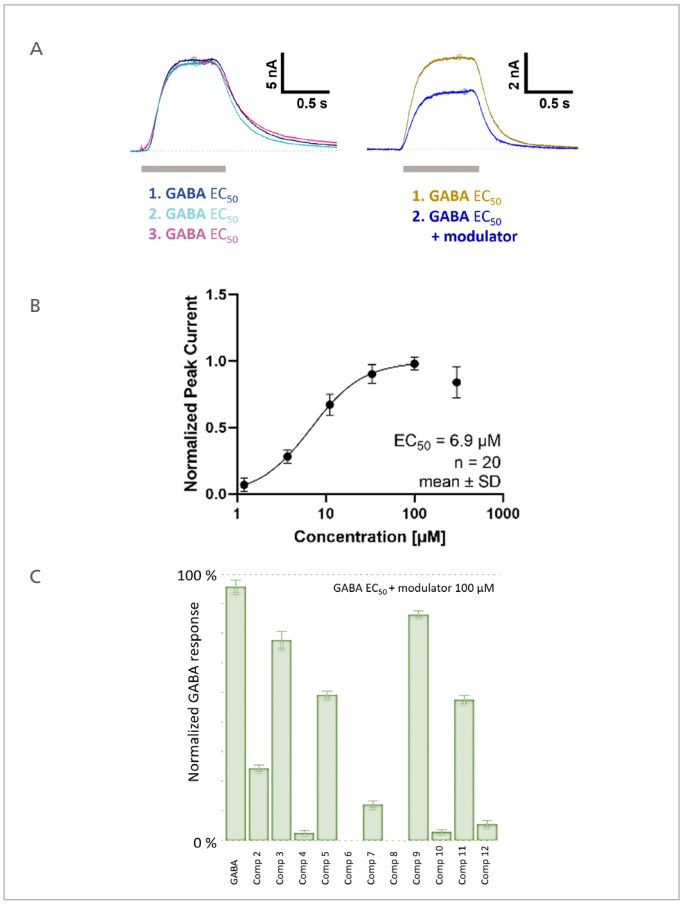


Fig. 1: A) Typical response to three repetitive exposures to GABA (EC_{50}) or GABA co-applied with a modulator. B) A GABA concentration-response curve recorded from a human-$GABA_A$ ($\alpha_5\beta_3\gamma_2$)-expressing HEK cell line. C) Bar graph illustrating % residual GABA response current (EC_{50}) when modulated by a high concentration of the different compounds (100 µM). Mean ± SD, n=10-16.

Next, we wanted to examine compound effects in primary hippocampal neurons, as the hippocampus is a brain region relevant to cognition and antipsychotic effects and where α_5-containing receptors are predominantly expressed. We, therefore, proceeded to electrophysiologically characterizing primary hippocampal neurons using the Sophion automated patch clamp systems (in Figure 2: K_V and Na_V current recordings, action potentials, assay success rates and representative current traces).

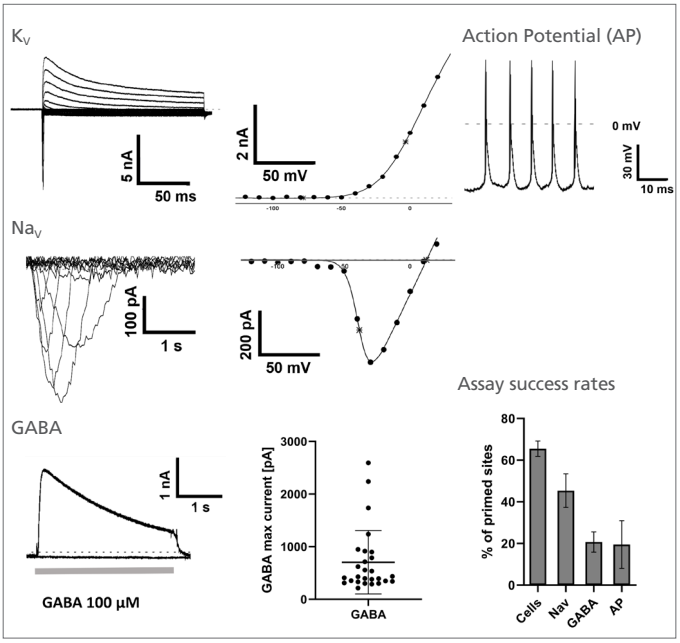


Fig. 2: Electrophysiological characterization of primary hippocampal neurons: **K_V** and **Na_V** current recordings in primary hippocampal neurons and matching I-V curves. The stars on the I-V curve indicate reversal potential and $V_{½}$. **GABA** currents elicited by 100 µM GABA. **Action Potentials** recorded in current clamp recording. **Assay success rates** describe the overall assay performance: We obtained a whole-cell success rate of 65% ± 6.5%. Among these cells, 69% ± 21% expressed sodium (Na_V) currents and out of the latter group, 44% ± 6% of cells showed an >100 pA response to 100 µM GABA. Furthermore, action potential firings were recorded from 31% of the cells that passed the quality criteria filter.

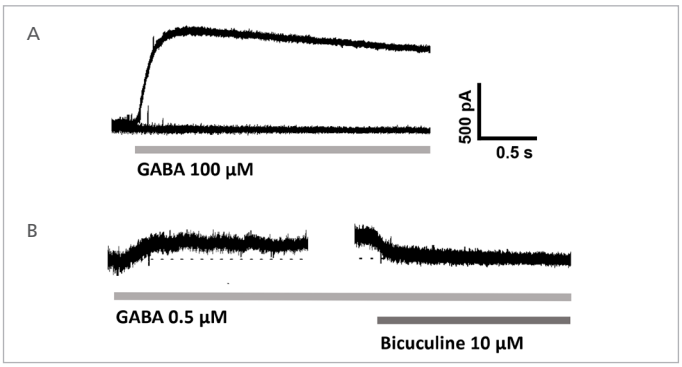


Fig. 3: A) Example trace of a current evoked by 100 µM GABA, recorded in a primary hippocampal neuron. B) Recording of the response to GABA 0.5 µM, in the same neuron as (A). The current was blocked by bicuculine (10 µM) revealing the size of GABA-mediated current.

Effects at GABA 0.5 µM plus 1, 10 or 100 µM compound were measured following a stable GABA response (after three repeats). Diazepam was used as a control. We observed strong inhibitory effects on the GABA-induced currents in the higher concentrations of several compounds, as expected based on the previous studies and consistent with our own HEK cell findings (Figure 3).

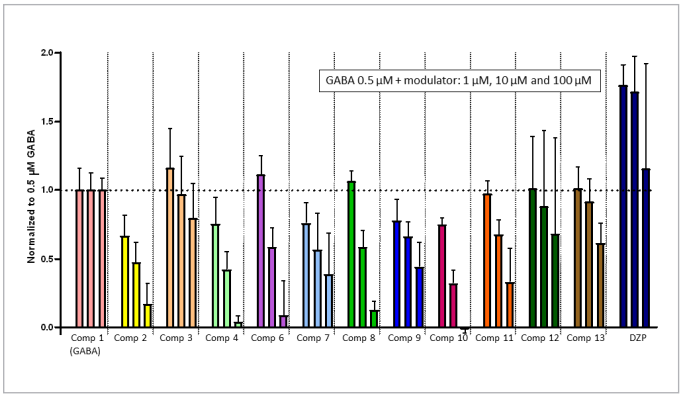


Fig. 4: Exploratory screen revealed GABA-effect of anti-psychotic drugs. The 12 compounds were co-applied with GABA (0.5 µM) similarly to evaluate their modulation. The compounds were added in increasing concentrations: 1 µM, 10 µM and 100 µM. Comp 1 (GABA) was added 3 times in 0.5 µM. DZP: Diazepam concentrations: 0.1 µM, 1 µM and 10 µM. Note: Due to handling issues, Comp 5 was excluded and replaced by Comp 13, which has similar structural relevance.

Conclusion

In our study, we characterized the effect of 12 antipsychotic compounds on $GABA_A$ ($\alpha_5\beta_3\gamma_2$) receptors stably expressed in HEK cells and found that a majority of the compounds had significant inhibitory effects on this receptor type.

Many of these compounds have often been studied in heterologous expression systems. Therefore, we additionally tested the effects of these compounds in primary hippocampal cultures in our automated patch clamp system. The compound effects were evaluated using sub-µM i.e. physiological extrasynaptic GABA concentrations, confirming relevant effects on the complex endogenous $GABA_A$ receptors of primary hippocampal neurons. Our results show a concentration-effect relationship, consistent with previous studies that have suggested a possible therapeutic relevance for the action of antipsychotic compounds on $GABA_A$ receptors (Bampali *et al.*, 2022; Manzo *et al.*, 2021).

Methods

- The cell line expressing human $GABA_A$ receptors was cultured according to the suppliers' description. ($\alpha_5\beta_3\gamma_2$)/HEK293 was kindly supplied by Charles River Laboratories, Cleveland, OH.
- All experiments were carried out at ambient temperature (controlled at 22°C ± 1°C) using Qube 384 multi-hole consumables and a standard whole-cell patch clamp protocol. The intracellular solution was CsF-based, with a Cl^- concentration of 10 mM (hence the outward currents).
- Hippocampal neurons are prepared from mice on embryonic day 16. Neuronal activity was measured after 14-16 days *in vitro* (DIV 14-16). Using an optimized cell dissociation protocol to obtain healthy cell membranes for patch-clamp experiments a whole-cell success rate of 65% ± 6.5% was obtained.
- All experiments were carried out at ambient temperature (controlled at 22°C ± 1°C) using Qube 384 single-hole consumables with high-resistance patch holes (R = 2.5). The intracellular solution was KF-based. For Na_V receptor recordings, extracellular [Na^+] was lowered to 10 mM.
- Data analysis was performed using the Sophion Analyzer Harrier Software (Sophion Bioscience A/S) and GraphPad Prism 9 (GraphPad Software Inc.).

For full dissociation protocol and example Qube 384 assays, please, contact your application scientist or info@sophion.com.

Acknowledgements

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