Ligand-activation of $GABA_A$ receptors on the automated patch clamp platforms QPatch and Qube 384 using conventional electrophysiology and optopharmacology

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Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS) and the binding of GABA to ionotropic GABA receptors ($GABA_AR$) is a crucial process in the healthy brain. An imbalance of GABA secretion or the malfunction of the receptor is associated with multiple disease areas like anxiety disorders, seizures and schizophrenia. Pharmacological manipulation of the receptor has therefore a large therapeutic potential, which is underscored by the amount of available treatment possibilities and the ongoing search for alternatives thereof[1-4].

$GABA_A$ receptors are ligand gated ion-channels that consist of 5 membrane spanning subunits[5,6] and are permeable to Cl^- ions. So far, 16 different subunits have been identified in humans (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, γ, θ, π) and their composition within the GABA receptor leads to different pharmacological responses[7]. Here, we show pharmacological modulation of the $GABA_AR$ using our high-throughput automated patch clamp (APC) systems QPatch and Qube 384. Our study includes a characterization of the heterogeneous $GABA_AR$ population of cultured primary hippocampal astrocytes and an evaluation of the $GABA_AR$ clone α5β3γ2. In addition, we utilize the well-characterized $GABA_AR$ response to establish a novel method for ligand release, namely the light-stimulated release of ruthenium-bipyridine-triphenylphosphine(RuBi)-caged GABA using light stimulation on the Qube 384 platform.

Conclusion

In our study we evaluated the pharmacological responses of the GABA receptor $GABA_A$ (α5β3γ2), stably expressed in a HEK cell line, and in the heterogeneous GABA receptor population of cultured primary hippocampal astrocytes, using the automated patch clamp platforms QPatch and Qube. Our results demonstrate the feasibility of performing $GABA_AR$-targeted drug-screening on our ACP platforms and introduces optopharmacology as a viable application possibility for high-throughput pharmacological experiments.

Materials and methods

Stably expressing HEK cells
$GABA_A$ (α5β3γ2)-HEK293 cells were kindly provided by Charles River Laboratories and cultured according to the supplier's description. All experiments were carried out at ambient temperature using QPatch or Qube multihole consumables (10 patch holes/well) and patched using a standard whole cell protocol.

Primary rat hippocampal astrocyte cultures
Hippocampi were isolated together with Saniona from P1-5 rat pups and astroglia-enriched cultures were grown according to Liu et al., 2003[8]. Electrophysiological experiments were carried out at ambient temperature using QPatch multihole consumables and physiological solutions.

Compound addition and incubation
All agents were evaluated in the presence of GABA in the concentration indicated. For QPatch compound application, the antagonist was applied prior to a 3 second application of GABA + antagonist. On the Qube, the GABA application duration was 0.8 seconds, delivered by liquid stacking.

Optopharmacology
RuBi-GABA from Tocris was applied and uncaged by a 475 nm light exposure.

Solutions
The extracellular solution contained (in mM): 145 NaCl, 10 HEPES, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$ (305 mOsm, pH 7.4), the intracellular solution 90 KCl, 50 KF, 11 EGTA, 10 HEPES, 4 Mg-ATP, 1 $MgCl_2$ (300 mOsm, pH 7.35).

Data Analysis
Analysis was performed using the Sophion Analyzer, Origin 7.5 (OriginLab Corporation) and GraphPad Prism 7.03 (GraphPad Software Inc.).

1. Pharmacology of $GABA_A$ (α5β3γ2)-HEK293 cells

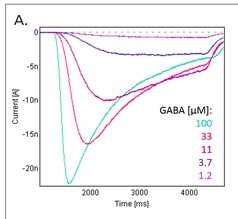

Fig. 1: Concentration-response relationship on the QPatch (cumulative)
A: Typical responses to the 3 sec application of GABA in increasing concentrations. B: Normalized response vs concentration (n = 32). The response was normalized to the current at the highest GABA concentration. Error bars: ± SD. EC_{50}: 12.2 µM.

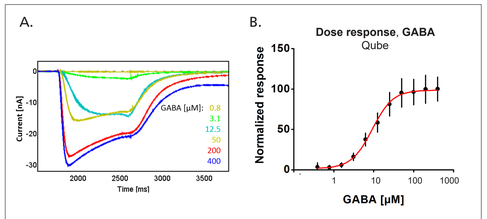

Fig. 2: **Concentration-response relationship on the Qube (non-cumulative)**
A: Typical responses to the 0.8 sec application of GABA in increasing concentrations. B: Normalized response vs concentration (n = min. 29 per concentration). The response was normalized to the average current at the highest GABA concentration. Error bars: ± SD. EC_{50}: 9.6 µM.

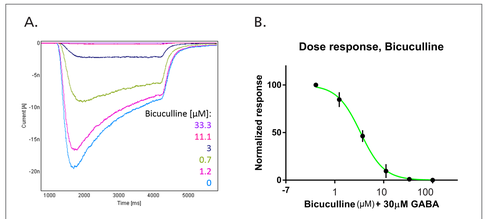

Fig. 3: Concentration-response relationship of bicuculline (QPatch)
A: Concentration-response relationship of the competitive antagonist bicuculline in the presence of 30 µM GABA. B: Plot of the normalized response vs concentration (n = 20). Error bars: ± SD. IC_{50} value at 30 µM GABA: 3.3 µM.

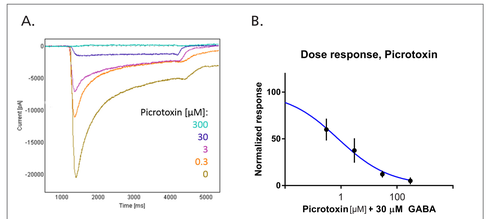

Fig. 4: **Concentration-response relationship of picrotoxin (QPatch)**
Overview of the 384-well A: The pore blocker picrotoxin was evaluated in the presence of 30 µM GABA. B: Normalized response vs concentration (n = 20). Error bars: ± SD. IC_{50} value: 0.8 µM.

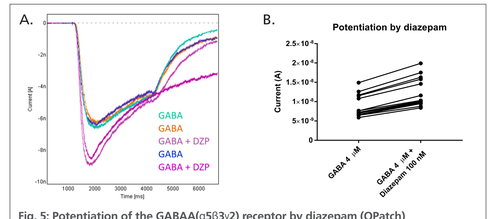

Fig. 5: Potentiation of the GABAA(α5β3γ2) receptor by diazepam (QPatch)
A: Typical traces of 4 µM GABA applied either on its own or in combination with 100 nM diazepam. Note the intentional lack of washout after the last drug application, hence the different shape of the trace. B: Individual increase in GABA-mediated current. Diazepam potentiated the GABA response in a reversible and reproducible manner (141% ± 16%, n = 17, p< 0.0001, paired students t-test). Paired recordings are connected.

2: Pharmacology of primary hippocampal astrocytes (QPatch)

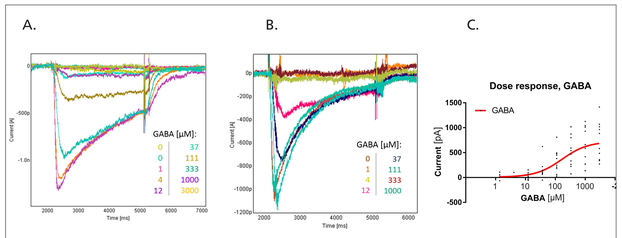

Fig. 6: Concentration-response relationship of GABA on hippocampal astrocytes
The cellular GABA response is conducted by a population of GABA receptors with different subunit composition and different pharmacology, and thus the response will be a population response. To evaluate the pharmacology of a physiologic GABA response, we employed primary cell cultures of rat hippocampal astrocytes. A and 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 due to the decreased signal to noise ratio.

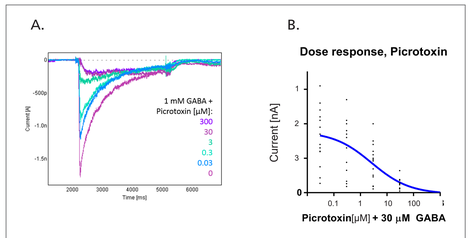

Fig. 7: Concentration-response relationship of picrotoxin on hippocampal astrocytes
A: Typical traces recorded from an astrocyte exposed to 1 mM GABA and increasing concentration of picrotoxin. B: Plot of current vs concentration (n = 13). IC_{50}: 2.2 µM ($CI_{95\%}$: 0.8 to 4.0 µM). The IC_{50} value for picrotoxin is consistent with what has been found for most $GABA_A$ receptors and with the current being picrotoxin-sensitive, the $GABA_A$ component of the current is confirmed.

3: Optopharmacology of $GABA_A$ (α5β3γ2)-HEK293 cells (Qube)

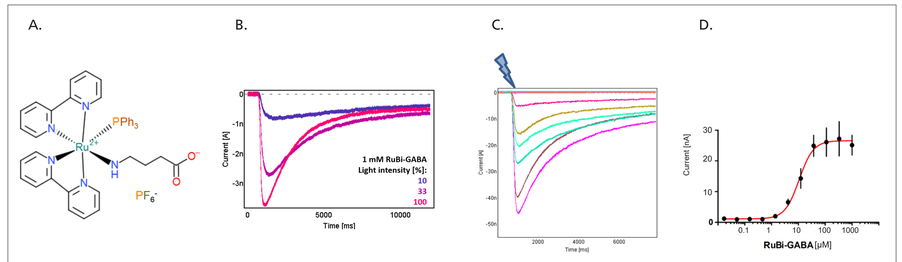

Fig. 8: RuBi-GABA application response
A: Chemical structure of Ruthenium-bipyridine-triphenylphosphine(RuBi)-caged GABA from Tocris (license from Columbia University). B: Light intensity-dependency of RuBi-GABA. C: Typical traces recorded after 200 msec light (475 nm) application in the presence of different concentrations of RuBi-GABA. D: Non-cumulative concentration-response curve for RuBi-GABA of a 3-fold dilution series. A Hill equation was fitted to the data and the calculated EC_{50} was 11 µM (n=32). Displayed are average values ± SD.

References

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