UNIVERSITY OF COPENHAGEN
DEPARTMENT OF DRUG DESIGN & PHARMACOLOGY

Electrophysiological and pharmacological characterization of $GABA_A$ receptor-mediated currents

Konstantina Bampali[1], Kim Boddum[2], Beatrice Badone[2], Anissa Bara[2], Heidi Marie Nielsen[1], Petrine Wellendorph[1]

[1]Department of Drug Design & Pharmacology, Faculty of Health & Medical Sciences, University of Copenhagen, Copenhagen Denmark
[2]Sophion Bioscience, Ballerup Denmark

Introduction

$GABA_A$ receptors constitute important inhibitory neurotransmitter receptors in the central nervous system and have a staggering variety of receptor subtypes and thus binding sites. Not only $GABA_A$ receptor targeting drugs such as benzodiazepines or sedative general anaesthetics elicit effects at these receptors by allosteric interaction sites, but a wide range of small molecules have been identified as $GABA_A$ receptor modulators, including multiple antipsychotic and antidepressant medications not intentionally targeting these receptors. Hippocampal dysfunction has long been considered to contribute to the pathophysiology of schizophrenia and post-mortem studies in the brains of patients with schizophrenia suggest that hippocampal expression of $GABA_A$ receptors is altered in a subtype-selective manner. The α5 $GABA_A$ receptor subunit, which is characterized by its relatively limited distribution and high abundance in the hippocampus, has been in the focus of clinical and preclinical schizophrenia research. The search for α5-containing subtype-preferring ligands has provided many compounds widely used in research. These molecules exert allosteric modulatory effects that can be GABA-induced current enhancement or reduction. Negative modulation of α5-containing $GABA_A$ receptors has been shown to promote hippocampal gamma oscillations, long-term potentiation, and learning, as well as have antidepressant effects associated with restored synaptic strength in the form of increased glutamatergic excitatory activity. In the 80s and 90s, the interactions of several antipsychotics with $GABA_A$ receptors have been considered serious candidates for eliciting part of the therapeutic effects (see studies from Squires & Saederup). Another key protein in neural plasticity, learning and memory is the calcium/calmodulin-dependent protein kinase II (CaMKII). γ-Hydroxybutyric acid (GHB) is a natural brain metabolite of GABA and CaMKIIα was recently found to be the GHB high-affinity target. $GABA_A$ receptors have also been studied as possible targets for GHB and analogue ligands. The role of $GABA_A$ receptors in mediating effects of GHB has been controversial.

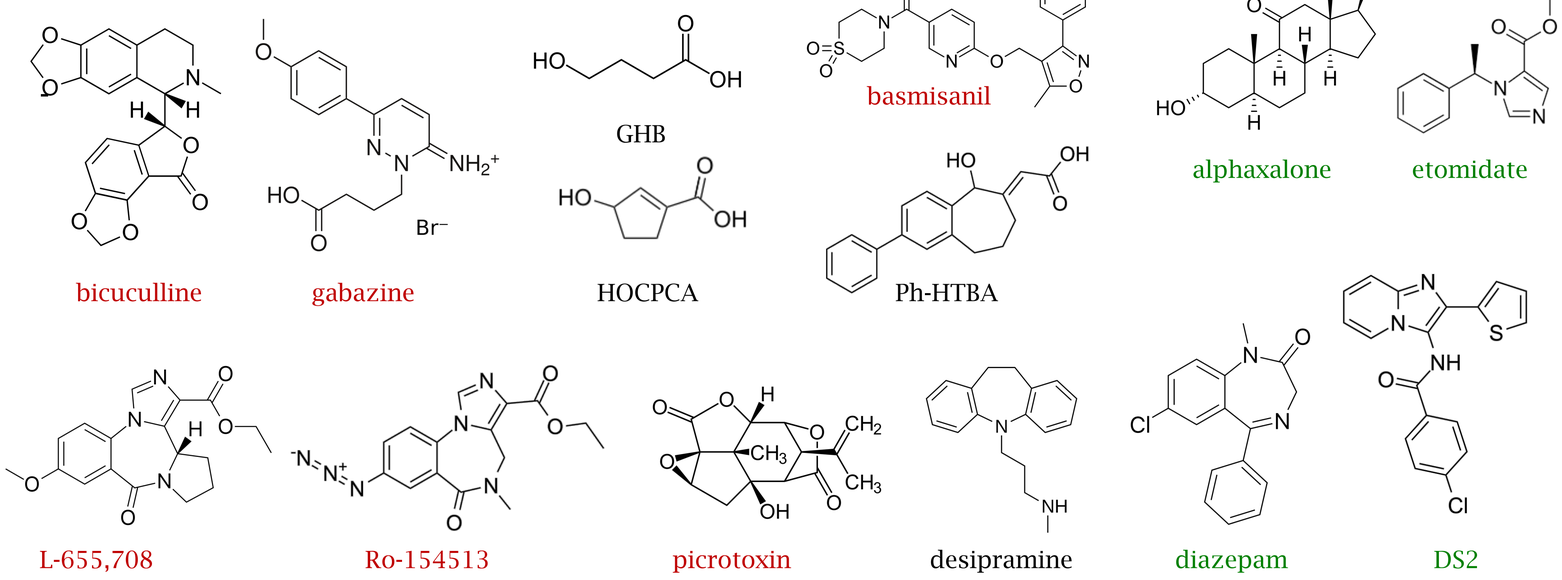
Aim

Here, we investigate a small library of tool compound effects on α5β3γ2 $GABA_A$ receptors, as well as GHB, HOCPCA and Ph-HTBA and the tricyclic antidepressant desipramine . Additionally, we aim to establish a protocol for measuring GABA currents in primary hippocampal neurons in the Sophion automated patch clamp systems and subsequently examine different compound effects.

Methods

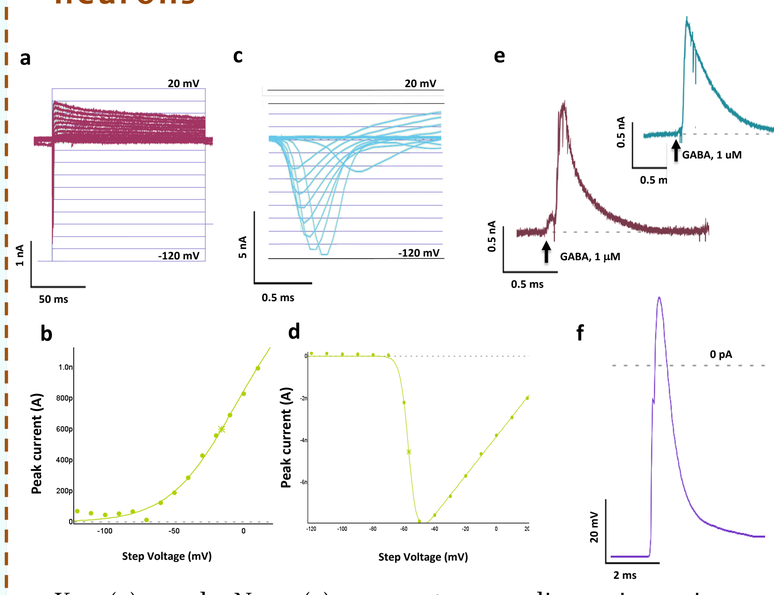
The cell line expressing human $GABA_A$ receptors were cultured according to the suppliers' description. (α5β3γ2)/HEK293 was kindly supplied by Charles River Laboratories, Cleveland, OH. All experiments were carried out at ambient temperature using Qube 384 multi-hole consumables and patched using a standard whole cell protocol. Intracellular solution is CsF based (hence the outward currents). Hippocampal neurons are prepared from mice at embryonic day 16. Neuronal activity was measured around DIV 14-16.
Data analysis was performed using the Sophion Assay Software and GraphPad Prism 9 (GraphPad Software Inc.).

Library of compounds



Library of compounds tested in this study. Marked with red are compounds that inhibit GABA-induced currents either by means of orthosteric antagonism, or via interaction with allosteric sites or via channel blocking and with green compounds that increase GABA-induced currents by positive allosteric modulation. Marked with black are compounds with unknown actions on α5β3γ2 $GABA_A$ receptors. More specifically, desipramine is a tricyclic antidepressant, while GHB, HOCPCA and Ph-HTBA are compounds that act, among other targets, on the CaMKIIα.

Recordings in primary hippocampal neurons

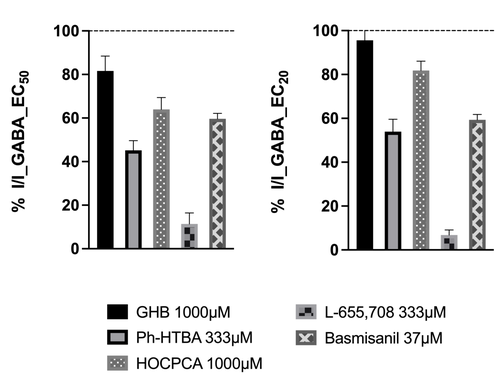


K_V (**a**) and Na_V (**c**) current recordings in primary hippocampal neurons. I-V curve of K_V (**b**) and Na_V (**d**) currents shown in (a) and (c), respectively. GABA currents elicited by 1µM GABA are depicted in (**e**). Current clamp recording showing an action potential is depicted in (**f**).

Compound effects at GABA EC_{80}

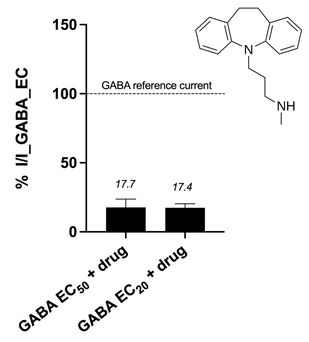
Concentration-dependent inhibition of GABA EC_{80} current at α5β3γ2 receptors by bicuculline, $ZnCl_2$, picrotoxin, Ro-154513, gabazine, as well as HOCPCA and Ph-HTBA. Data are depicted as mean ± SEM.

Compound effects at GABA EC_{50} & EC_{20}



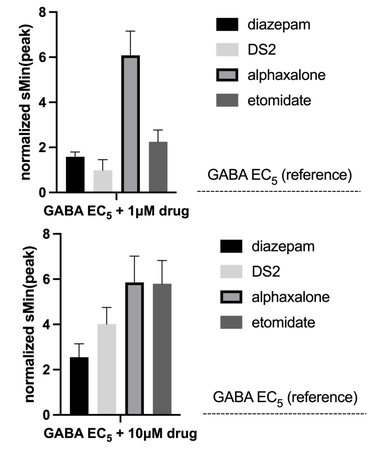
[a]The bar graphs illustrate % modulation of currents elicited by an EC_{20} and EC_{50} GABA concentration by a high concentration of the compounds. The dotted line is used to visualize the baseline (100%) of control current. Mean ± SEM, n=4-7

Desipramine



The graph illustrates % negative modulation of currents elicited by an EC_{50} and EC_{20} GABA concentration by 333µM desipramine.
Mean ± SEM, n=4

Compounds at GABA EC_5



Normalized sMin (peak) responses of diazepam, DS2, alphaxalone and etomidate elicited by an EC_5 GABA concentration. Mean ± SEM, n=16-22

Conclusion & Outlook

Here, we show that tool compound effects on α5β3γ2 $GABA_A$ receptors expressed in HEK293 cells can be reproduced using Qube and Qpatch II automated patch clamp systems. We showed that the CaMKIIα ligands HOCPCA and Ph-HTBA can also negatively modulate these receptors when co-applied with three different GABA concentrations. GHB showed minimal to no effect on that receptor subtype. Interestingly, the tricyclic antidepressant desipramine strongly inhibited GABA-induced currents both at GABA EC_{50} and EC_{20}. Positive allosteric modulators also exerted their effects as expected. Last but not least, after many optimization steps, we were able to use primary hippocampal cultures with automated patch clamp systems and observe GABA-mediated currents. Next, we will further examine the effects of CaMKIIα ligands as well as additional tricyclic antidepressant and antipsychotic compounds on hippocampal neurons and α5β3γ2 $GABA_A$ receptors.

References: Squires & Saederup (1988, 1993, 1997, 1998, 2000); Bampali *et al.* (2022) https://doi.org/10.1111/bph.15807; Absalom et al. (2012) https://doi.org/10.1073/pnas.1204376109; Leurs *et al.* (2021) https://doi.org/10.1073/pnas.2108079118; Lu *et al.* (2023) https://doi.org/10.1038/s41380-023-01982-8