Optimizing methods for the functional screening at $GABA_A$ receptor subtypes

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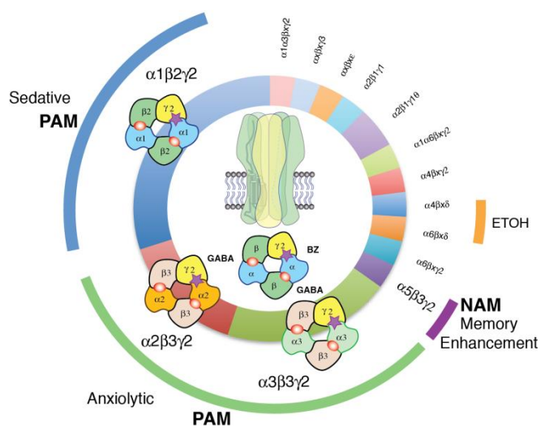
1 – Introduction

With 19 genes encoding for separate protein subunits and the capacity to form distinct pentameric receptors by combining multiple subunits, the $GABA_A$ receptors is one of the most versatile ligand-gated ion channel in the central nervous system. The rich repertoire of possibilities offered by $GABA_A$ receptors is magnificently exploited in the central nervous system with the fine tuning of synaptic receptors and the high sensitivity of extrasynaptic receptors.

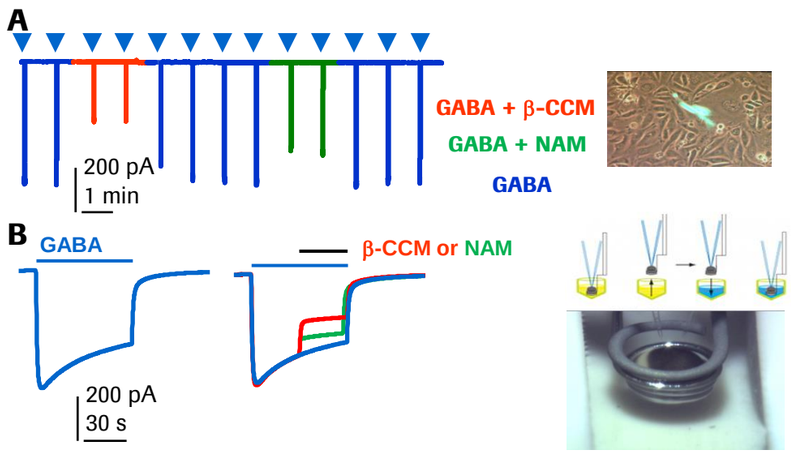
The therapeutic potential of $GABA_A$ specific molecules is illustrated by the importance of benzodiazepines and other related molecules in neurological treatments. Whereas exploration of large libraries of compounds ranging up to several hundred thousand is often conducted using binding or functional assays using optical signal detection, successive steps in drug development pathways requires a precise characterization of the physiological and pharmacological properties of the lead compounds that is often tested using electrophysiological means.

Taking advantage of automated electrophysiological recordings of recombinant $GABA_A$ receptors expressed in *Xenopus* oocytes and expression into HEK293 cells, we have examined the contribution of the γ and ε subunits at α1 and α2 containing receptors. As a first step, the properties of heteromeric receptors comprising only α and β subunits were compared to that of receptors comprising an additional γ1, γ2 or ε subunit. Subsequently, effects of prototypal allosteric modulators were tested at the different receptor combinations in binding and function.

2 – $GABA_A$ receptor subtypes



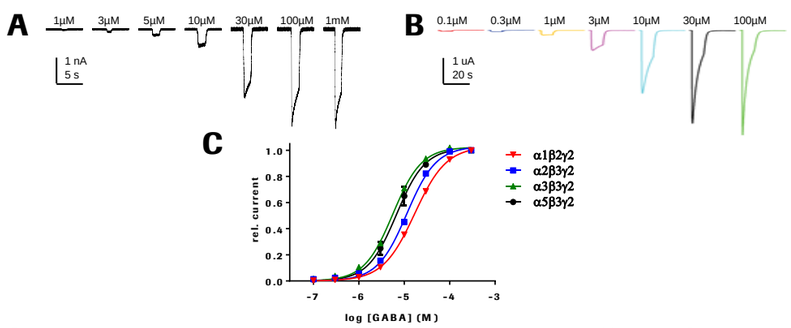
3 – Efficacy assessment in HEK293 cells and *Xenopus* Oocytes



A HEK293 cell lines stably expressing $GABA_A$ receptor subunit combinations were recorded using the whole-cell mode of the patch-clamp technique. GABA (blue arrows) was applied to the investigated cell for 1 s every 1 min in the absence and presence of β-CCM or a negative allosteric modulator (NAM).

B *XENOPUS* oocytes were microinjected with RNA solution mixes coding for the $GABA_A$ receptor subunit combinations and recorded in voltage-clamp using a HiClamp device. GABA (blue bar) was applied alone or in the presence of a drug.

4 – GABA sensitivity is dependent on the subunit composition

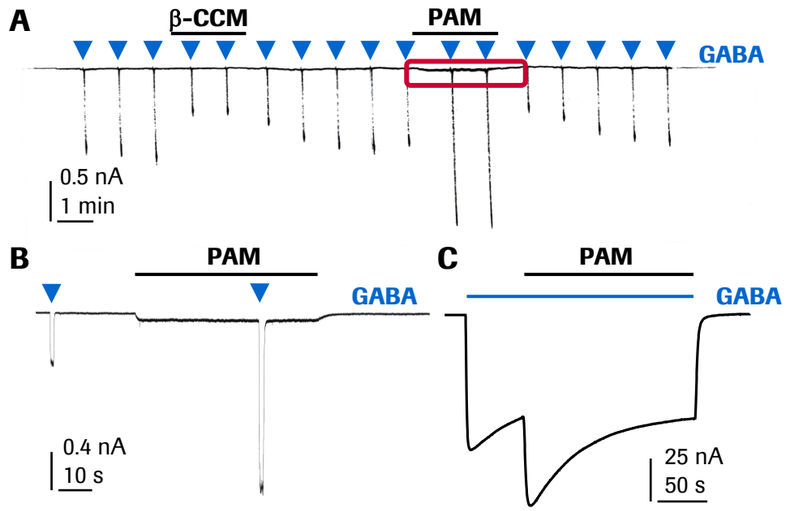


GABA concentration response curves from HEK293 cells (A) and *Xenopus* Oocytes (B) expressing the α5β3γ2 $GABA_A$ receptor subunit combination. Increasing concentrations were applied for 1 s (A) or 10s (B). (C) Concentration-response curves reveal the contribution of the α-subunit to GABA sensitivity.

Contribution of γ1, γ2 and ε subunits

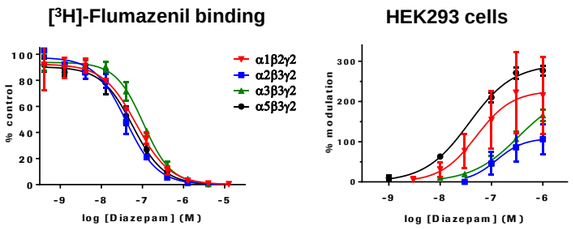
	EC_{50}	nH
α1β2	3.50 ±0.09	1.28±0.02
α1β2γ2	48.50±1.20	1.50±0.00
α1β2ε	1.83±0.38	0.71±0.04
α2β2	4.99±0.40	1.31±0.08
α2β2γ2	76.00±7.33	1.49±0.07
α2β2γ1	43.20±6.85	1.08±0.03

5 – A positive allosteric modulator (PAM) activates currents w/o GABA in a HEK293 cell expressing α5β3γ2 receptors



A, B GABA (blue arrows) in the presence or absence of drugs (black bars) was repetitively applied to the investigated HEK293 cell for 1 s in 1 min intervals using a multi-barreled micro-applicator pipette.
C The PAM-induced current cannot be detected with the protocol used for *Xenopus* oocytes recordings.

6 – Diazepam binding / function correlation in HEK293 cells



	Binding Ki (nM)	Function EC_{50} (nM)
α1β2γ2	34.8	47.5
α2β3γ2	21.4	128
α3β3γ2	51.4	234
α5β3γ2	17.2	36.5

7 – Conclusions

Comparison of results obtained at receptors with different compositions offers additional possibilities to explore the role of the different subunit interfaces within a receptor complex on the overall physiological and pharmacological properties.

By expending our knowledge on the properties of a broader repertoire of $GABA_A$ receptors, these studies are further bridging the gap between our understanding of the contribution of specific receptors and certain brain functions and illustrate the importance of thorough functional characterization.