

Potency of GABA at human recombinant GABA_A receptors expressed in *Xenopus* oocytes: a mini review

Nasiara Karim · Petrine Wellendorph ·
 Nathan Absalom · Graham A. R. Johnston ·
 Jane R. Hanrahan · Mary Chebib

Received: 23 December 2012 / Accepted: 26 December 2012 / Published online: 6 February 2013
 © Springer-Verlag Wien 2013

Abstract GABA_A receptors are members of the ligand-gated ion channel superfamily that mediate inhibitory neurotransmission in the central nervous system. They are thought to be composed of 2 alpha (α), 2 beta (β) subunits and one other such as a gamma (γ) or delta (δ) subunit. The potency of GABA is influenced by the subunit composition. However, there are no reported systematic studies that evaluate GABA potency on a comprehensive number of subunit combinations expressed in *Xenopus* oocytes, despite the wide use of this heterologous expression system in structure–function studies and drug discovery. Thus, the aim of this study was to conduct a systematic characterization of the potency of GABA at 43 human recombinant GABA_A receptor combinations expressed in *Xenopus* oocytes using the two-electrode voltage clamp technique. The results show that the α-subunits and to a lesser extent, the β-subunits influence GABA potency. Of the binary and ternary combinations with and without the γ2L subunit, the α6/γ2L-containing receptors were the most sensitive to GABA, while the β2- or β3-subunit conferred higher sensitivity to GABA than receptors containing the β1-subunit

with the exception of the α2β1γ2L and α6β1γ2L subtypes. Of the δ-subunit containing GABA_A receptors, α4/δ-containing GABA_A receptors displayed highest GABA sensitivity, with mid-nanomolar concentrations activating α4β1δ and α4β3δ receptors. At α4β2δ, GABA had low micromolar activity.

Keywords GABA · Synaptic and extrasynaptic GABA_A receptors · GABA potency · *Xenopus* oocytes · Two-electrode voltage clamp

Introduction

γ-Aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the vertebrate central nervous system (CNS) mediates fast synaptic transmission via ionotropic GABA_A receptors. These receptors are pentameric assemblies of individual subunits arranged around a central, chloride-conducting pore. To date, 16 human GABA_A receptor subunit genes have been characterized and grouped together according to their amino acid similarity and termed: α1–6, β1–3, γ1–3, δ, ε, θ, and π (Bonnert et al. 1999; Barnard et al. 1998; Sinkkonen et al. 2000). Of the six α-subunits, α1–3 are localized at synapses, while α4–6 subunits exist at sites outside the synapse (Belelli et al. 2009), and have higher sensitivity to GABA (Storustovu and Ebert 2006). The activation of GABA_ARs by GABA is therefore not restricted to fast synaptic transmission alone, and is in part dependent on subunit composition.

The γ2-containing GABA_A receptors can be found at both synaptic and extrasynaptic sites. These γ2-subunits combine with α1–3,5-subunits to form receptors that are sensitive to benzodiazepines (Rudolph et al. 2001; Atack et al. 2005). Each α-subunit confers different physiological

N. Karim · N. Absalom · G. A. R. Johnston ·
 J. R. Hanrahan · M. Chebib (✉)
 Faculty of Pharmacy A15, University of Sydney,
 Sydney, NSW 2006, Australia
 e-mail: maryc@pharm.usyd.edu.au;
 mary.collins@sydney.edu.au

N. Karim
 Department of Pharmacy, University of Malakand,
 Chakdara, Dir (Lower), KPK, Pakistan

P. Wellendorph
 Department of Drug Design and Pharmacology, Faculty
 of Health and Medical Sciences, University of Copenhagen,
 2 Universitetsparken, DK-2100 Copenhagen, Denmark

characteristics, and displays differential regional expression profiles in the brain. The role of each α -subunit in relation to benzodiazepine pharmacology has been elucidated using genetically modified mice in which a genetic mutation was introduced to individual α -subunits in order to render the receptor subtype insensitive to diazepam. These studies found that the sedative, anterograde, amnesic, and partly the anticonvulsant actions of diazepam were mediated by $\alpha 1$ -containing GABA_A receptors, the anxiolytic-like effects were mediated by $\alpha 2$ -containing GABA_A receptors, and the myorelaxant action was mediated in part by $\alpha 3$ - and $\alpha 5$ -containing GABA_A receptors. Moreover, the development of tolerance to the sedative action of benzodiazepines was linked to $\alpha 5$ -containing GABA_A receptors, while the addictive properties have been linked to $\alpha 1$ -containing GABA_A receptors (Rudolph et al. 2001).

The δ -subunit predominates on peri- and extrasynaptic locations (Nusser et al. 1998; Wei et al. 2003). Preferentially forming receptors with $\alpha 4$ - or $\alpha 6$ -subunits, the $\alpha 6/\delta$ -containing receptors are located to cerebellar granule cells, while the $\alpha 4/\delta$ -containing receptors are located to the dentate gyrus, thalamus, and neostriatum (Barnard et al. 1998). In addition to these combinations, binary GABA_A receptors composed of α - and β -subunits are thought to also exist in the CNS.

Determining which of these combinations has a distinct physiological role in the CNS has been hampered by the lack of subtype selective agents. Studies using a variety of techniques including in situ hybridization, immunoprecipitation and immunocytochemistry studies, and the development of transgenic mice, have enabled one to infer the likeliest native GABA_A receptor subtypes involved in distinct physiological roles (Wisden et al. 1992; Pirker et al. 2000; Korpi et al. 2002; Sperk et al. 1997; Hutcheon et al. 2004; Olsen and Sieghart 2008). However, given there is a high level of structural diversity of potential GABA_A receptor subtypes, the impact of this diversity on GABA potency is not well known (Olsen and Sieghart 2008; Sieghart 2006). Indeed, many subunit combinations can form functional receptors when different subunits are expressed in vitro, and these consist of binary combinations composed from α - and β -subunits, ternary combinations composed of α -, β -, and either γ - or δ -subunits, and in some cases quaternary combinations exist (Sieghart 2006). Further impacting on this complex set of combinations, the effect of stoichiometry remains to be evaluated systematically. As a result of this diversity, the sensitivity to GABA must differ given the different receptor locations and other biochemical conditions. In a recent study, Mortensen et al. (2012) evaluated the effect of GABA expressed in human embryonic kidney (HEK293) cells to try to address this issue. This study was quite comprehensive and serves as a valuable reference for GABA responses in this cellular

expression system. Given that choice of cell expression system contributes to the potency of GABA and other ligands, and that there is a large body of evidence that the host heterologous expression system influences the function of the resulting receptors at the cell membrane (Palma et al. 2003), the current study attempts to supplement with pharmacological data obtained using another highly used cell expression system, namely the *Xenopus laevis* oocyte expression system. The oocyte expression system is a very robust and widely used expression system to evaluate the effects of agents acting on GABA_A receptors, but surprisingly little is known about the differing effects of GABA on these receptors. The current study and mini review provides data on the potency of GABA at 43 human GABA_A receptor subtypes composed of $\alpha 1$ –6, $\beta 1$ –3 alone and in combination with $\gamma 2L$ - or δ -subunits expressed in *Xenopus* oocytes.

Materials and methods

GABA and buffer supplements were all purchased from Sigma (St. Louis, MO, USA).

GABA receptor subunit constructs

Human $\alpha 1$, $\alpha 2$, $\beta 2$ and $\gamma 2L$ DNA in pcDM8 were provided by Dr. Paul Whiting (Merck, Sharpe and Dohme Research Labs, Harlow, UK). $\alpha 3$ and $\beta 3$ in pGEMHE, $\alpha 5$ in pcDNA3, $\alpha 4$ and δ in pcDNA1/Amp, and $\alpha 6$ and $\beta 1$ in pcDM8 were a gift from Prof Bjarke Ebert (H. Lundbeck A/S, Valby, Denmark). cDNA vectors were linearized with the appropriate restriction endonucleases (Table 1) and capped transcripts were produced from linearized plasmids using the 'mMessage mMachine' T-7 transcript kit from Ambion (Austin, TX, USA). The quality of cRNA was determined by 0.5 % agarose gel electrophoresis. mRNA concentrations were measured by NanoDrop® ND-1000 UV–Vis Spectrophotometer. cRNA was diluted with nuclease-free water and stored at -80°C .

Expression of recombinant GABA receptors in *Xenopus* oocytes

Oocyte preparation

The methods for oocyte harvesting and preparation have been described previously (Karim et al. 2011). In brief, stage V–VI oocytes were sorted and injected (Nanoject, Drummond Scientific Co., Broomall, PA, USA) with cRNA reconstituted in nuclease-free water in a ratio of 1:1:10 for $\alpha:\beta:\gamma$, 5:1:5 for $\alpha:\beta:\delta$ and 1:1 for $\alpha:\beta$ receptors. Oocytes were incubated for 4–8 days in standard

Table 1 The pcDNA vector, restriction digestion enzyme and mRNA polymerase used for each receptor subunit

Receptor subunit	Plasmid	Restriction enzyme	RNA polymerase
α1	pcDM8	NotI	T7
α2	pcDM8	NotI	T7
α3	pGEMHE	NheI	T7
α4	pcDNA1/Amp	HpaI	T7
α5	pcDNA3	NotI	T7
α6	pcDM8	SapI	T7
β1	pcDM8	HpaI	T7
β2	pcDM8	NotI	T7
β3	pGEMHE	NheI	T7
γ2L	pcDM8	NotI	T7
δ	pcDNA1/Amp	HpaI	T7

sterile-filtered ND96 solution (96 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 1.8 mM CaCl₂, 5 mM HEPES, pH 7.4), supplemented with theophylline (0.5 mM), pyruvate (5 mmol/L), gentamycin (50 µg/mL) and 2 % horse serum at 18 °C.

Electrophysiology

Currents were recorded using the two-electrode voltage clamp (TEVC) technique as described elsewhere (Hall et al. 2005). Oocytes were individually placed in a 150 µl chamber connected to a reservoir bottle containing ND96 solution. Glass microelectrodes were made with a tip resistance of 0.2–1.2 MΩ using a micropipette puller (Narishige Scientific Instrument Laboratory, Tokyo, Japan) and filled with 3 M KCl (0.5–2 MΩ). The oocytes were impaled and the membrane potential was clamped at –60 mV while continuously superfused with ND96 solution. Current amplitudes were calculated off-line using Chart software v3.6 (ADInstruments, NSW, Australia).

Responses to GABA were normalized as $I\% = (I/I_{\max}) \times 100$, where I is the peak amplitude of current response and I_{\max} is the maximal current produced by GABA measured in each individual cell. Normalized responses were pooled and graphed as the mean \pm SEM from at least three oocytes from at least two different batches. Responses were fitted to the four-parameter logistic equation: $I = I_{\max} / (1 + (EC_{50}/[A])^{n_H})$; where I is the peak amplitude of the current elicited by a given concentration of agonist $[A]$, I_{\max} is the maximum amplitude of the current, EC_{50} is the concentration required for half-maximal response, and n_H is the Hill coefficient (Prism v5 GraphPad software, San Diego, CA, USA). All statistical calculations are presented as means standard error of the

mean (SEM) or as mean [95 % confidence intervals (CI)]. Groups were compared using one-way ANOVA followed by Tukey's or Dunnett's post hoc test.

Results

Validation of expression of αβγ and αβδ receptors in *Xenopus* oocytes

With GABA-response curves for ternary receptor constructs, it is ideal that little to no current being measured is flowing through αβ binary receptors. Zn²⁺ has been reported to display distinct inhibitory effects at αβ- versus αβγ-containing receptors (Smart et al. 1991; Storustovu and Ebert 2006; Hosie et al. 2003); thus, we initially investigated the effects of zinc ions (Zn²⁺) at representative GABA_A receptor subtypes. In all combinations tested, we evaluated the effect of Zn²⁺: at α1β2 receptors, 10 and 100 µM Zn²⁺ inhibited approximately 40 \pm 8 % and 70 \pm 14 % of the current elicited by GABA (100 µM), respectively, whereas at α1β2γ2L receptors, there was no inhibition by 10 or 100 µM Zn²⁺ (Fig. 1a, b, respectively) indicating that the γ2L subunit was incorporated. At α1β3 receptors, 0.1 and 1 µM Zn²⁺ inhibited approximately 55 \pm 5 % and 80 \pm 12 % of the current elicited by GABA (30 µM), respectively ($n = 3$; Fig. 1c), whereas at α1β3δ receptors, Zn²⁺ did not inhibit the current produced by GABA (10 µM; Fig. 1d, $n = 4$), implicating that the δ-subunit was incorporated (ANOVA followed by Dunnett's post hoc test; $n = 4$; $p > 0.05$). Similarly, at α4β3 receptors, Zn²⁺ (0.1 and 1 µM) inhibited GABA (10 µM) by 55 \pm 10 % and 95 \pm 5 % respectively ($n = 4$; Fig. 1e), whereas at α4β3δ receptors, Zn²⁺ (0.1 and 1 µM) inhibited GABA (3 µM) by 35 \pm 9 % and 40 \pm 6 %, respectively ($n = 6$; Fig. 1f), indicating a mixed population of receptors (Karim et al. 2012). Thus, Zn²⁺ was used throughout the study to demonstrate incorporation of the γ- and δ-subunits (data not shown).

Current ranges elicited by a maximum GABA concentration at the various GABA_A receptor subtypes

The maximal current amplitude for GABA when tested at different αβ combinations ranged from 150 to 750 nA for α1β1–3, 100–350 nA for α2β1–3, 100–470 nA for α3β1–3, 80–500 nA for α4β1–3, 100–390 nA for α5β1–3, and 500–3000 nA for α6β1–3. Similarly, the maximal current amplitudes for different αβγ2L ranged from 300 to 4000 nA for α1β1–3γ2L, 290–1200 nA for α2β1–3γ2L, 200–3500 nA for α3β1–3γ2L, 350–4000 nA for α4β1–3γ2L, 300–740 nA for α5β1–3γ2L, and 3500–7000 nA for α6β1–3γ2L. The maximal current amplitude ranged

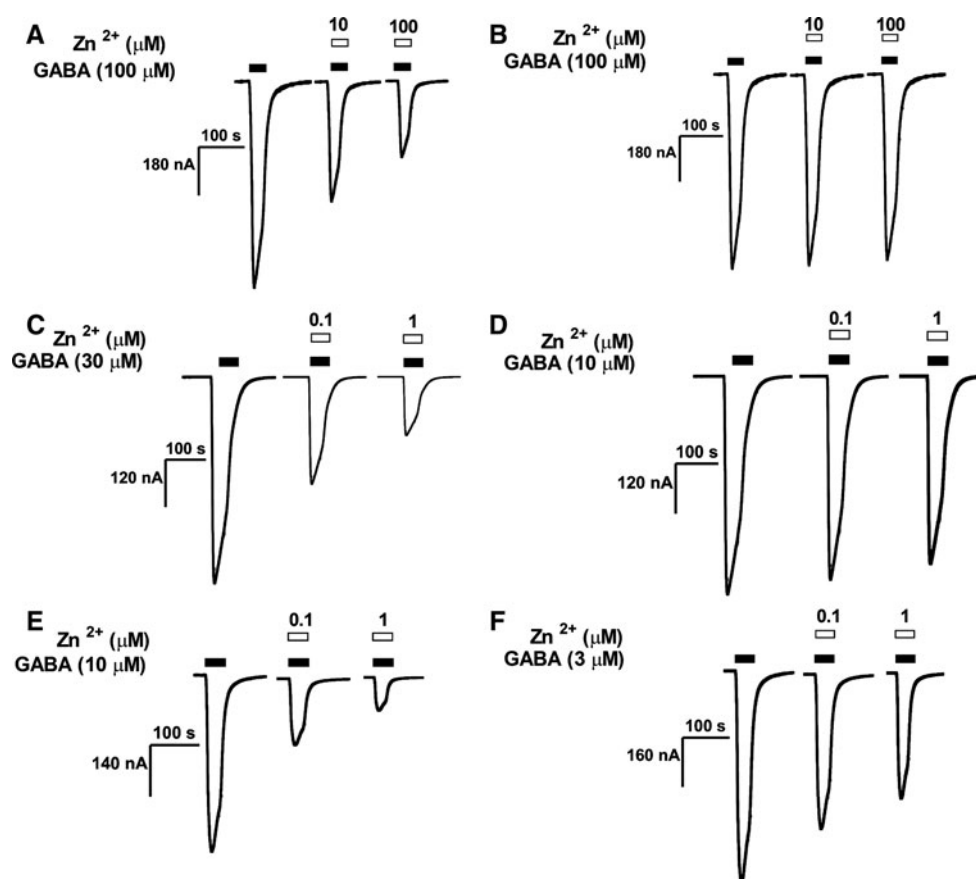


Fig. 1 Example of a recording from oocytes illustrating the blockade that Zn^{2+} exerts on the GABA response on $\alpha 1\beta 2$, $\alpha 1\beta 3$ and $\alpha 4\beta 3$ receptors expressed in *Xenopus* oocytes. **a, b** Current traces (nA vs. s) illustrating the blockade that Zn^{2+} exerts on the GABA response on $\alpha 1\beta 2$ and $\alpha 1\beta 2\gamma 2\text{L}$ receptors. Zn^{2+} (10 and 100 μM) inhibited GABA (100 μM) by $40 \pm 8\%$ and $70 \pm 14\%$, respectively, at $\alpha 1\beta 2$ receptors but did not inhibit GABA responses at $\alpha 1\beta 2\gamma 2\text{L}$ receptors. **c, d** Zn^{2+} (0.1 and 1 μM) inhibited GABA (30 μM) by $55 \pm 5\%$ and

$80 \pm 12\%$, respectively, at $\alpha 1\beta 3$ receptors but did not inhibit GABA responses (10 μM) at $\alpha 1\beta 3\delta$ receptors. **e, f** Example of a trace showing the effect of Zn^{2+} inhibiting GABA at $\alpha 4\beta 3$ and $\alpha 4\beta 3\delta$ receptors. Zn^{2+} (0.1 and 1 μM) inhibited GABA (10 μM) by $55 \pm 10\%$ and $95 \pm 5\%$, respectively, at $\alpha 4\beta 3$ and (0.1 and 1 μM) inhibited GABA (3 μM) by $35 \pm 9\%$ and $40 \pm 6\%$, respectively, at $\alpha 4\beta 3\delta$ receptors

250–750, 500–1000, 550–4000, 500–4500, 3500–6500, 3500–6600, and 3500–7000 nA for $\alpha 1\beta 3\delta$, $\alpha 4\beta 1\delta$, $\alpha 4\beta 2\delta$, $\alpha 4\beta 3\delta$, $\alpha 6\beta 1\delta$, $\alpha 6\beta 2\delta$, and $\alpha 6\beta 3\delta$, respectively. The maximum chloride amplitude for each individual oocyte is dependent on the number of receptors expressed on the oocyte membrane, as well as the potential difference across the membrane, the chloride concentrations inside and outside the cell, and the intrinsic properties of the receptors including the single channel conductance and maximum open channel probability of the receptor. As such, it is not a reliable comparison of the efficiency of receptor expression at the cell surface for each subtype, but gives a guide to the relative ease of acquiring data for each subtype using the oocyte expression system.

Effect of GABA at $\alpha 1$ – $\beta 1$ and $\alpha 1$ – $\beta 1\gamma 2\text{L}$ combinations

For studies of GABA_A binary receptor combinations, all α -subunits were expressed sequentially with $\beta 1$ –subunits

and for ternary combinations sequentially with $\beta 1$ –3- and $\gamma 2\text{L}$ -subunits. The concentration–response curves for GABA at the $\alpha 1$ – $\beta 1$ –3 and at $\alpha 1$ – $\beta 1$ –3 $\gamma 2\text{L}$ receptors are shown in Fig. 2. GABA potency for each receptor combination was compared by determining the individual EC_{50} value. The EC_{50} and n_H values are summarized in Table 2. As previously reported, GABA was more potent on the binary combinations ($\alpha 1$ – $\beta 1$ –3) than on the ternary combinations ($\alpha 1$ – $\beta 1$ –3 $\gamma 2\text{L}$) (Ducic et al. 1995).

At the binary combinations containing the $\beta 1$ -subunit, GABA was most potent on $\alpha 4\beta 1$ and $\alpha 6\beta 1$ compared to other combinations (Fig. 2a, b). The EC_{50} values varied between 0.7 and 268 μM . The apparent order of potency for the binary receptor subtypes is $\alpha 4\beta 1 > \alpha 6\beta 1 > \alpha 5\beta 1 > \alpha 2\beta 1 > \alpha 1\beta 1 > \alpha 3\beta 1$. The Hill coefficient ranged 0.6–1.5 for binary combinations ($\alpha 1$ – $\beta 1$ –3) (Table 2). At the ternary GABA_A receptor combinations, the EC_{50} values varied between 18 and 260 μM , with GABA being the most potent at the $\alpha 6\beta 1\gamma 2\text{L}$ receptor subtype (Fig. 2c, d).

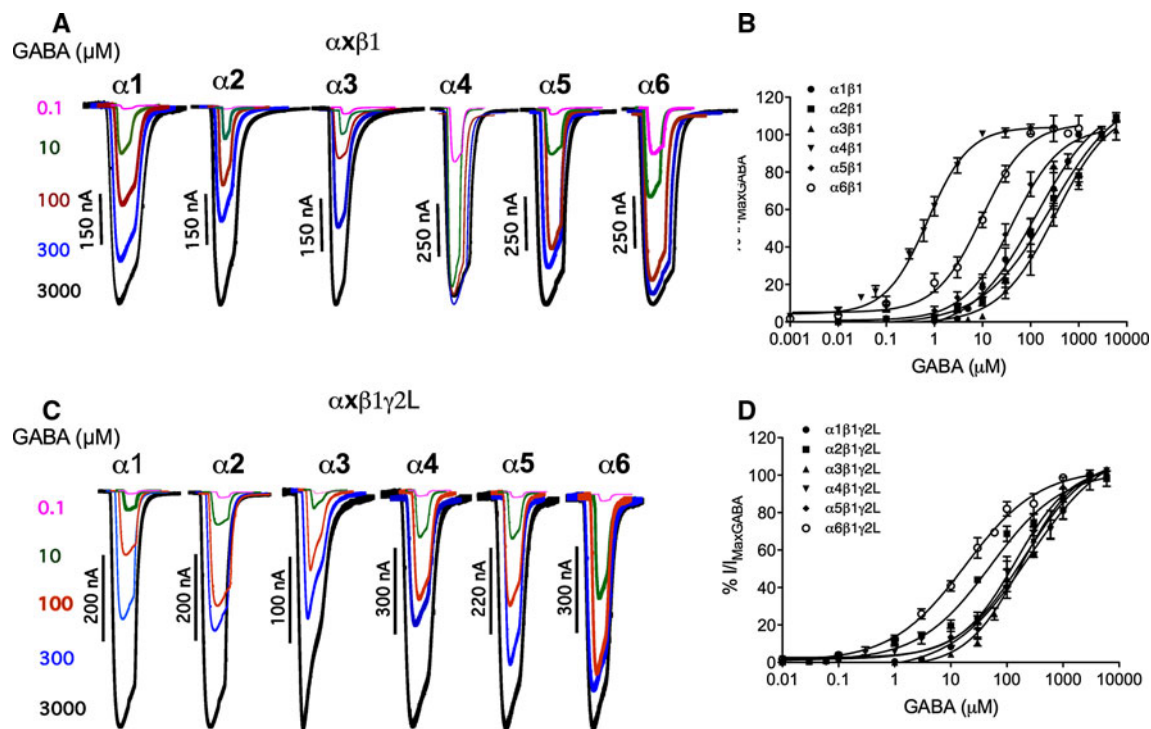


Fig. 2 Assessing the impact of α -subunits and the $\beta 1$ -subunit alone, and with the $\gamma 2\text{L}$ -subunit on GABA potency. **a** Superimposed membrane currents activated by GABA concentrations ranging from 0.1 μM to 3 mM for $\alpha 1$ – $\alpha 6\beta 1$ GABA_A receptors expressed in *Xenopus* oocytes. **b** Dose–response curves for GABA at $\alpha 1\beta 1$ (filled circle) $\alpha 2\beta 1$ (filled square) $\alpha 3\beta 1$ (filled upward triangle) $\alpha 4\beta 1$ (filled downward triangle) $\alpha 5\beta 1$ (filled diamond) and $\alpha 6\beta 1$ (open circle) receptor subtypes expressed

in *Xenopus* oocytes. **c** Superimposed membrane currents activated by GABA concentrations ranging from 0.1 μM to 3 mM for $\alpha 1$ – $\alpha 6\beta 1\gamma 2\text{L}$ GABA_A receptors expressed in *Xenopus* oocytes. **d** Dose–response curves for GABA at $\alpha 1\beta 1\gamma 2\text{L}$ (filled circle), $\alpha 2\beta 1\gamma 2\text{L}$ (filled square), $\alpha 3\beta 1\gamma 2\text{L}$ (filled upward triangle), $\alpha 4\beta 1\gamma 2\text{L}$ (filled downward triangle), $\alpha 5\beta 1\gamma 2\text{L}$ (filled diamond) and $\alpha 6\beta 1\gamma 2\text{L}$ (open circle) receptor subtypes expressed in *Xenopus* oocytes. Data are expressed as mean \pm SEM ($n = 3$ – 10)

The order of potency for GABA at different ternary receptor subtypes is $\alpha 6\beta 1\gamma 2\text{L} > \alpha 5\beta 1\gamma 2\text{L} > \alpha 2\beta 1\gamma 2\text{L} > \alpha 4\beta 1\gamma 2\text{L} > \alpha 3\beta 1\gamma 2\text{L} > \alpha 1\beta 1\gamma 2\text{L}$. The Hill coefficient ranged 0.6–0.9 for ternary combinations ($\alpha 1$ – $\alpha 6\beta 1$ – $\gamma 2\text{L}$) (Table 2).

Effect of GABA at $\alpha 1$ – $\alpha 6\beta 2$ and $\alpha 1$ – $\alpha 6\beta 2\gamma 2\text{L}$ combinations

At the binary combinations containing the $\beta 2$ -subunit, GABA was found to be more potent on $\alpha 4\beta 2$ and $\alpha 6\beta 2$ combinations. The EC_{50} values for $\alpha 1$ – $\alpha 6\beta 2$ receptors varied between 2 and 56 μM (Table 2). The apparent order of potency is $\alpha 6\beta 2 > \alpha 4\beta 2 > \alpha 1\beta 2 \approx \alpha 5\beta 2 > \alpha 2\beta 2 \approx \alpha 3\beta 2$ (Fig. 3a, b; Table 2). The Hill coefficient ranged 0.6–1.3 for $\alpha 1$ – $\alpha 6\beta 2$ binary subtypes. The EC_{50} values for GABA at ternary receptor combinations containing the $\beta 2$ -subunit ranged from 25 to 133 μM . The apparent order of potency at these GABA_A receptor subtypes is $\alpha 6\beta 2\gamma 2\text{L} > \alpha 5\beta 2\gamma 2\text{L} > \alpha 4\beta 2\gamma 2\text{L} > \alpha 1\beta 2\gamma 2\text{L} > \alpha 2\beta 2\gamma 2\text{L} > \alpha 3\beta 2\gamma 2\text{L}$ (Fig. 3c, d; Table 2). The Hill coefficient ranged from 0.8 to 1.1 for $\alpha 1$ – $\alpha 6\beta 2$ binary subtypes (Table 2).

Effect of GABA at $\alpha 1$ – $\alpha 6\beta 3$ and $\alpha 1$ – $\alpha 6\beta 3\gamma 2\text{L}$ combinations

At the binary combinations containing the $\beta 3$ -subunit, GABA was again found to be more sensitive compared to their ternary receptor subtype counterparts (Table 2). The EC_{50} values varied between 0.4 and 90 μM at the binary receptor combinations. GABA was most sensitive to the $\alpha 4\beta 3$ subtype with an EC_{50} in the nanomolar range (0.41 μM), followed by $\alpha 6\beta 3$ with an EC_{50} of 1.6 μM (Fig. 4a, b; Table 1). The apparent order of potency at the binary subtypes was as follows $\alpha 4\beta 3 \approx \alpha 6\beta 3 > \alpha 5\beta 3 > \alpha 1\beta 3 > \alpha 2\beta 3 \approx \alpha 3\beta 3$. The Hill coefficient for $\alpha 1$ – $\alpha 6\beta 3$ subtypes ranged from 0.5 to 0.9 (Table 2).

GABA activated the ternary combinations with similar potencies ($p > 0.05$: ANOVA followed by Tukey's multiple comparison post hoc test), and the EC_{50} values varied between 23 and 157 μM (Fig. 4c, d). The apparent order of potency for GABA is $\alpha 6\beta 3\gamma 2\text{L} > \alpha 5\beta 3\gamma 2\text{L} > \alpha 3\beta 3\gamma 2\text{L} \approx \alpha 4\beta 3\gamma 2\text{L} > \alpha 1\beta 3\gamma 2\text{L} \approx \alpha 2\beta 3\gamma 2\text{L}$ (Table 2). The Hill coefficient ranged 0.8–1.1 for $\alpha 1$ – $\alpha 6\beta 1$ – $\gamma 2\text{L}$ ternary subtypes (Table 2).

Table 2 Agonist properties of GABA across α , β and γ GABA_A receptor subunits

Receptor subtype	GABA EC ₅₀ (μ M) (95 % CI)	n_H	n
$\alpha 1\beta 1$	141 (51.9–381)	0.7 ± 0.3	4
$\alpha 2\beta 1$	45.4 (16.6–49.2)	1.5 ± 0.7	3
$\alpha 3\beta 1$	268 (146–491.)	0.6 ± 0.3	3
$\alpha 4\beta 1$	0.72 (0.60–0.80)	1.0 ± 0.2	5
$\alpha 5\beta 1$	41.8 (29.8–58.5)	0.9 ± 0.2	5
$\alpha 6\beta 1$	7.7 (5.0–11.6)	1.2 ± 0.2	4
$\alpha 1\beta 1\gamma 2L$	259 (179–375)	0.7 ± 0.3	6
$\alpha 2\beta 1\gamma 2L$	51.3 (32.6–80.7)	0.8 ± 0.5	4
$\alpha 3\beta 1\gamma 2L$	249 (64.2–193)	0.6 ± 0.5	5
$\alpha 4\beta 1\gamma 2L$	80 (51.5–127)	0.9 ± 0.2	6
$\alpha 5\beta 1\gamma 2L$	31.1 (23.4–41.2)	0.7 ± 0.3	6
$\alpha 6\beta 1\gamma 2L$	18 (13.2–25.1)	0.8 ± 0.4	5
$\alpha 1\beta 2$	22 (14.4–32.5)	0.7 ± 0.3	6
$\alpha 2\beta 2$	56 (26.9–119)	0.6 ± 0.3	3
$\alpha 3\beta 2$	56.2 (34.8–90.7)	0.7 ± 0.5	3
$\alpha 4\beta 2$	2.29 (1.54–3.40)	0.9 ± 0.3	4
$\alpha 5\beta 2$	23.6 (14.6–38.0)	0.6 ± 0.3	5
$\alpha 6\beta 2$	2 (1.4–2.8)	1.3 ± 0.3	4
$\alpha 1\beta 2\gamma 2L$	107 (74.2–156)	1.0 ± 0.2	8
$\alpha 2\beta 2\gamma 2L$	131 (93.8–182)	0.8 ± 0.3	10
$\alpha 3\beta 2\gamma 2L$	133 (88–201)	1.1 ± 0.2	6
$\alpha 4\beta 2\gamma 2L$	103 (61.9–173)	0.8 ± 0.3	6
$\alpha 5\beta 2\gamma 2L$	41.1 (34.2–49.2)	1.0 ± 0.2	5
$\alpha 6\beta 2\gamma 2L$	25 (18.8–33.2)	0.9 ± 0.3	6
$\alpha 1\beta 3$	34.5 (23.4–50.8)	0.8 ± 0.3	3
$\alpha 2\beta 3$	89.7 (44.3–182)	0.7 ± 0.4	3
$\alpha 3\beta 3$	90.7 (44.3–183)	0.7 ± 0.5	4
$\alpha 4\beta 3$	0.41 (0.25–0.69)	0.6 ± 0.1	8
$\alpha 5\beta 3$	14.6 (10.4–20.4)	0.8 ± 0.3	5
$\alpha 6\beta 3$	1.7 (0.8–3.5)	0.5 ± 0.2	4
$\alpha 1\beta 3\gamma 2L$	156 (96.0–252)	0.9 ± 0.2	5
$\alpha 2\beta 3\gamma 2L$	157 (94.9–259)	0.8 ± 0.4	5
$\alpha 3\beta 3\gamma 2L$	127 (81.7–206)	0.9 ± 0.2	4
$\alpha 4\beta 3\gamma 2L$	127 (68.2–236)	1.0 ± 0.2	6
$\alpha 5\beta 3\gamma 2L$	33.3 (22.4–49.3)	0.6 ± 0.5	5
$\alpha 6\beta 3\gamma 2L$	23.3 (15.5–34.9)	0.9 ± 0.3	8

In summary, the binary combinations containing $\alpha 1$ – $\alpha 6\beta 1$ –3 have higher GABA potencies compared to the ternary combinations containing $\alpha 1$ – $\alpha 6\beta 1$ –3 $\gamma 2L$.

Effect of GABA at recombinant GABA_A receptors containing the δ -subunit

$\alpha 4\beta \delta$ and $\alpha 6\beta \delta$ receptors constitute the majority of extrasynaptic GABA_A receptors (Boileau et al. 2002). Recently, we have investigated the effect of GABA at various δ -subunit containing GABA_A receptors (Karim et al.

2012). Table 3 summarises the results from that study. Figure 5a shows the examples of GABA traces at $\alpha 4\beta 1\delta$, $\alpha 4\beta 2\delta$, $\alpha 6\beta 1\delta$, and $\alpha 6\beta 3\delta$ GABA_A receptors displaying mono-phasic concentration response curves. In contrast, $\alpha 4\beta 3\delta$ (injected as a 5:1:5 mRNA ratio) and $\alpha 6\beta 2\delta$ receptors displayed bi-phasic concentration response curves highlighting high and low affinity sites (Fig. 5b, c). GABA displayed the highest potency for $\alpha 4\beta 1\delta$ (EC₅₀ = 0.024 μ M), while potency of GABA at $\alpha 4\beta 2\delta$, $\alpha 6\beta 1\delta$, and $\alpha 6\beta 3\delta$ did not significantly differ ($p > 0.05$; ANOVA followed by Tukey's post hoc test), and varied between 0.35 and 1 μ M (Fig. 5d; Table 3).

Discussion

The pharmacological properties observed among the various native GABA_A receptor subtypes are most likely related to specific mechanisms regulating receptor subunit expression (Wisden et al. 1992). Without selective agonists or antagonists, it is almost impossible to determine the properties of each native GABA_A receptor subtype and identify which of the potential combinations exist, in vivo, let alone their changes in spatial and temporal expression.

Analysis of the effect of GABA at recombinant GABA_A receptors has previously been described, but in many cases, for only a limited number of receptor subtypes or for different cell expression systems (Ducic et al. 1995; Kleingoor et al. 1993; Knoeflach et al. 1996; Mortensen et al. 2012). As the cell expression system influences the effect of GABA, the current study focused on the *Xenopus* oocyte expression system because this system is extensively used for a number of structure–function studies and in drug discovery. A total of 43 human GABA_A receptors were expressed in the oocyte system and the effect of GABA was evaluated using TEVC methods.

In receptors consisting of binary α - and β -subunits, or ternary α -, β - and $\gamma 2$ -subunits, the potency of GABA varied, influenced mainly by the type of α -subunit. In general, binary receptor subtypes were more sensitive to GABA than their $\gamma 2$ -containing counterparts. Binary GABA_A receptors composed of $\beta 2$ - and $\beta 3$ -subunits did not affect GABA potency. GABA was most potent at receptors containing $\alpha 4$ - and $\alpha 6$ -subunits followed by $\alpha 5$ - and $\alpha 1$ -subunits. The least sensitive receptors to GABA were those composed of $\alpha 2$ - and $\alpha 3$ -containing receptors. In contrast, binary receptors composed of $\beta 1$ -subunit, GABA was most potent at $\alpha 4$ - and $\alpha 6$ -containing receptors but least potent at $\alpha 3$ - and $\alpha 1$ -containing receptors.

The marked differences in GABA potency may, in part, be related to the receptor stoichiometry. Indeed, the potency of acetylcholine can differ depending on the number of α - versus β -subunits that make up the

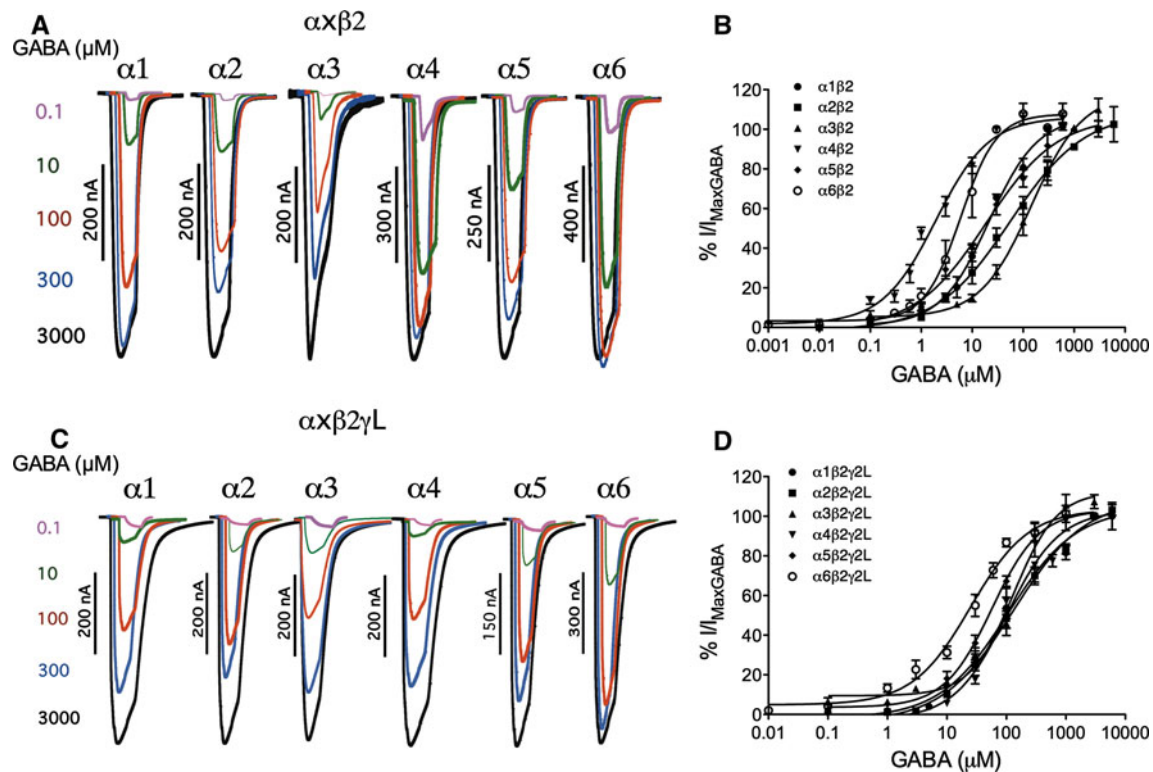


Fig. 3 Assessing the impact of α -subunits and $\beta 2$ -subunit alone, and with $\gamma 2\text{L}$ -subunit on GABA potency. **a** Superimposed membrane currents activated by GABA concentrations ranging from 0.1 μM to 3 mM for $\alpha 1\text{--}6\beta 2$ GABA_A receptors expressed in *Xenopus* oocytes. **b** Dose-response curves for GABA at $\alpha 1\beta 2$ (filled circle) $\alpha 2\beta 2$ (filled square) $\alpha 3\beta 2$ (filled upward triangle) $\alpha 4\beta 2$ (filled downward triangle) $\alpha 5\beta 2$ (filled diamond) and $\alpha 6\beta 1$ (open circle) receptor subtypes expressed in *Xenopus*

oocytes. **c** Superimposed membrane currents activated by GABA concentrations ranging from 0.1 μM to 3 mM for $\alpha 1\text{--}6\beta 2\gamma 2\text{L}$ GABA_A receptors expressed in *Xenopus* oocytes. **d** Dose-response curves for GABA at $\alpha 1\beta 2\gamma 2\text{L}$ (filled circle), $\alpha 2\beta 2\gamma 2\text{L}$ (filled square), $\alpha 3\beta 2\gamma 2\text{L}$ (filled upward triangle), $\alpha 4\beta 2\gamma 2\text{L}$ (filled downward triangle), $\alpha 5\beta 2\gamma 2\text{L}$ (filled diamond) and $\alpha 6\beta 2\gamma 2\text{L}$ (open circle) receptor subtypes expressed in *Xenopus* oocytes. Data are expressed as mean \pm SEM ($n = 3\text{--}10$)

heteromeric nicotinic acetylcholine (nACh) receptor (Harpsoe et al. 2011; Wu et al. 2006). Furthermore, Wagoner and Czajkowski (2010) show that differences exist in the expression ratios of α - versus β -subunits for $\alpha 1\beta 2$ and $\alpha 4\beta 2$ GABA_A receptors. In their study, a 3:2 stoichiometry was identified for $\alpha 1\beta 2$, while for $\alpha 4\beta 2$ GABA_A receptors, the 2:3 stoichiometry was formed. How such differences in receptor stoichiometry could influence GABA potency is not known.

Our results and those of others (Mortensen et al. 2012; Ducic et al. 1995; Knoeflach et al. 1996) show that among the ternary $\gamma 2\text{L}$ -containing receptor subtypes, the $\alpha 6$ -containing receptors are the most sensitive to GABA, followed by the $\alpha 5$ -containing receptors. In contrast to data obtained using HEK293 cells (Mortensen et al. 2012), we find that the least sensitive receptors are those composed of $\alpha 1\text{--}4$ -containing receptors. Using oocytes, GABA potency did not significantly differ between $\alpha 1\text{--}4$ -containing receptors, while using HEK293 cells, the $\alpha 2$ - and $\alpha 3$ -containing receptors were significantly less potent to GABA, a trend we observed with the binary but not the ternary

combinations. This difference may be due to the expression system used and to the type of $\gamma 2$ -subunit used. The $\gamma 2$ -subunit exists in two splice variants, termed $\gamma 2\text{S}$ and $\gamma 2\text{L}$. The long-splice variant ($\gamma 2\text{L}$) differs from the short variant ($\gamma 2\text{S}$) by the inclusion of eight additional amino acids in the intracellular loop between TM3 and TM4. In this study, we used the $\gamma 2\text{L}$ -subunit, while Mortensen and colleagues used the $\gamma 2\text{S}$ -subunit. The variability may be related to a difference in phosphorylation. The additional amino acids within the TM3 and TM4 loop contain an extra phosphorylation site, which may under certain conditions influence the GABA potency (Brandon et al. 2000).

At $\gamma 2\text{L}$ -containing receptors, the $\beta 2$ - or $\beta 3$ -subunits did not have a major impact on GABA potency. However, in $\beta 1$ -containing receptors, GABA potency varied in a similar manner to their binary counterparts, i.e., GABA did not significantly differ among the $\alpha 2$ -, $\alpha 4\text{--}6$ -subunits, while the least sensitive receptors to GABA were the $\alpha 1$ - and $\alpha 3$ -containing receptors.

The $\gamma 2\text{L}$ -subunit did not affect GABA potency, as there is no GABA binding site located on this subunit. In

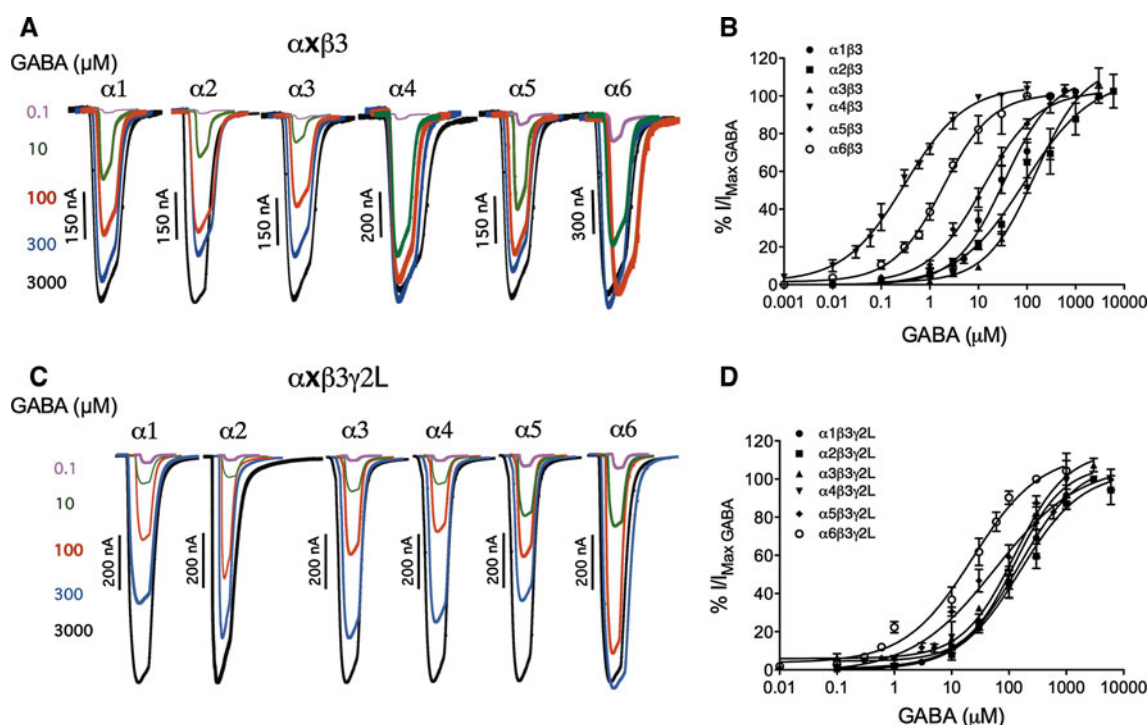


Fig. 4 Assessing the impact of α -subunits and $\beta 3$ -subunit alone, and with $\gamma 2\text{L}$ -subunit on GABA potency. **a** Superimposed membrane currents activated by GABA concentrations ranging from 0.1 μM to 3 mM for $\alpha 1-6\beta 3$ GABA_A receptors expressed in *Xenopus* oocytes. **b** Dose-response curves for GABA at $\alpha 1\beta 3$ (filled circle) $\alpha 2\beta 3$ (filled square) $\alpha 3\beta 3$ (filled upward triangle) $\alpha 4\beta 3$ (filled downward triangle) $\alpha 5\beta 3$ (filled diamond) and $\alpha 6\beta 3$ (open circle) receptor subtypes expressed in *Xenopus*

oocytes. **c** Superimposed membrane currents activated by GABA concentrations ranging from 0.1 μM to 3 mM for $\alpha 1-6\beta 3\gamma 2\text{L}$ GABA_A receptors expressed in *Xenopus* oocytes. **d** Dose-response curves for GABA at $\alpha 1\beta 3\gamma 2\text{L}$ (filled circle), $\alpha 2\beta 3\gamma 2\text{L}$ (filled square), $\alpha 3\beta 3\gamma 2\text{L}$ (filled upward triangle), $\alpha 4\beta 3\gamma 2\text{L}$ (filled downward triangle), $\alpha 5\beta 3\gamma 2\text{L}$ (filled diamond) and $\alpha 6\beta 3\gamma 2\text{L}$ (open circle) receptor subtypes expressed in *Xenopus* oocytes. Data are expressed as mean \pm SEM ($n = 3-10$)

contrast, the δ -subunit had a major impact on GABA potency. δ -Containing GABA_A receptors were highly sensitive to GABA, activating $\alpha 4\beta \delta$ and $\alpha 6\beta \delta$ GABA_A subtypes in the nanomolar range (Karim et al. 2012). The potency for GABA was also highly influenced by the type of β -subunit. When $\beta 1$ - and $\beta 3$ -subunits are combined with $\alpha 4/\delta$ -subunits, GABA displayed low nanomolar potency, while at the $\alpha 4\beta 2\delta$ combination, GABA had micromolar potency. In contrast, GABA had low nanomolar activity with the $\alpha 6\beta 2\delta$, while activated $\alpha 6\beta 1\delta$ and $\alpha 6\beta 3\delta$ with high nanomolar concentrations.

Interestingly, at $\alpha 6\beta 2\delta$ and $\alpha 4\beta 3\delta$ GABA_A subtypes, the effects of GABA preferentially fitted a two-site model. This indicates that mixed populations of receptors are being formed when the oocyte system is used (Hadley and Amin 2007; Karim et al. 2012). Varying the ratio of cRNA injected into the oocyte, an approach extensively used with nACh receptors, varied the population of receptors being expressed (Hadley and Amin 2007; Karim et al. 2012). Whether these receptors exist as different stoichiometric forms as evidenced from concatenated receptors (Kaur et al. 2009; Sigel et al. 2009) or whether there is “contamination” of $\alpha 4\beta 3$ receptors (Meera et al. 2011; Karim et al. 2012), as evidenced by Zn^{2+}

Table 3 Effect of GABA at δ -subunit containing GABA_A receptors (Karim et al. 2012)

Receptor subtype	GABA EC ₅₀ (μM) (95 % CI)	n_{H}	n
$\alpha 1\beta 3\delta$	8.7 (6.4–11.7)	0.8 ± 0.3	4
$\alpha 4\beta 1\delta$	0.024 (0.019–0.030)	1.1 ± 0.1	8
$\alpha 4\beta 2\delta$	1.00 (0.89–1.31)	1.3 ± 0.2	4
$\alpha 4\beta 3\delta$ biphasic 5:1:5 injection ratio	EC ₅₀ (1) = 0.012 (0.006–0.025) EC ₅₀ (2) = 1.3 (0.7–2.5)	n_{H} (1) = 1.2 ± 0.5 (2) = 1.1 ± 0.3	24
$\alpha 4\beta 3\delta$ monophasic	0.016 (0.014–0.018)	0.8 ± 0.1	5
$\alpha 6\beta 1\delta$	0.35 (0.24–0.52)	0.9 ± 0.3	6
$\alpha 6\beta 2\delta$	EC ₅₀ (1) = 0.05 (0.02–0.15) EC ₅₀ (2) = 8.7 (3.7–20)	n_{H} (1) = 0.9 ± 0.3 (2) = 1.7 ± 1.0	4
$\alpha 6\beta 3\delta$	0.44 (0.33–0.53)	0.9 ± 0.4	10

inhibition of higher GABA concentrations is yet to be determined. Interestingly, when using HEK cells, only the low affinity $\alpha 4\beta 3\delta$ receptors are expressed.

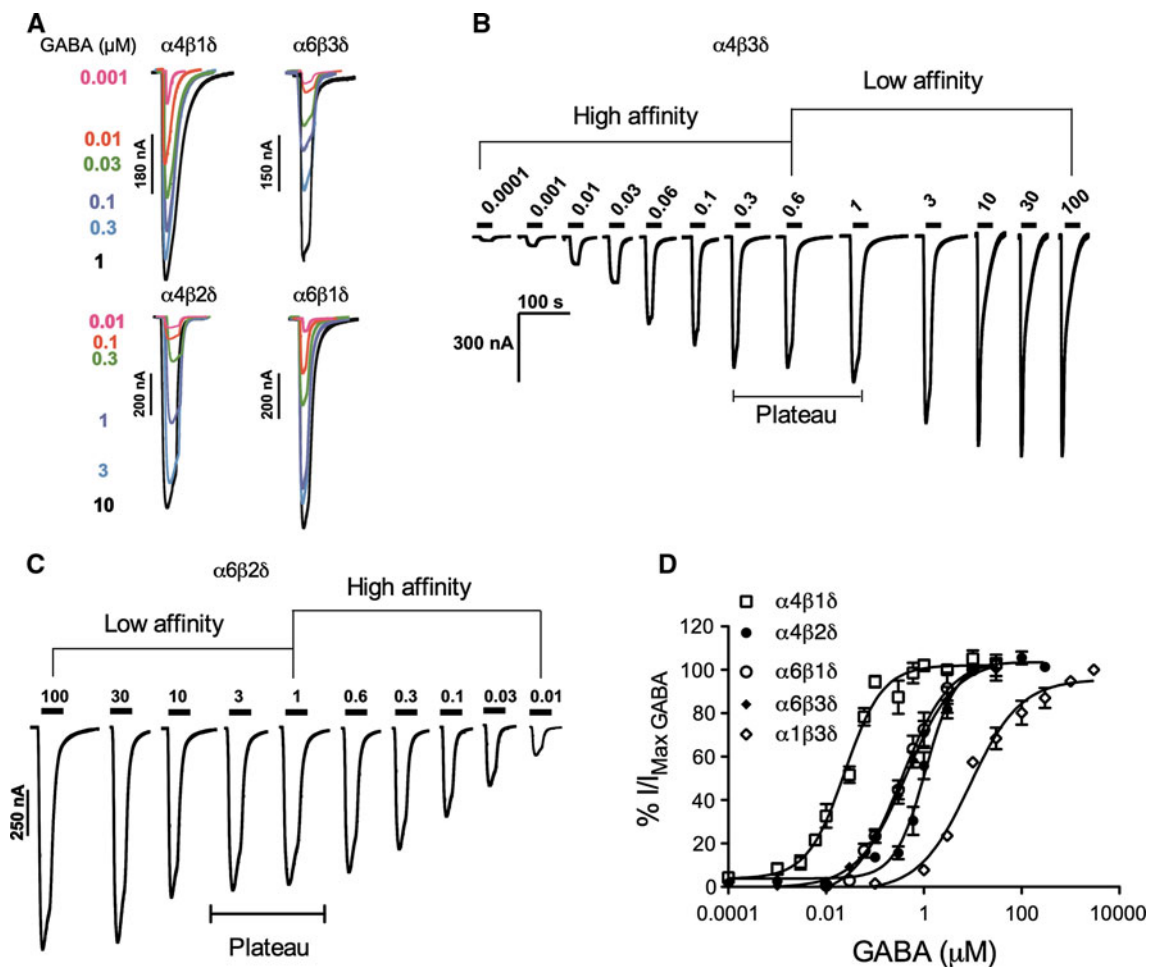


Fig. 5 Assessing the impact of δ GABA_A receptors subunit on GABA potency. Representative traces showing membrane currents activated by various GABA concentrations at **a** $\alpha 4\beta 1\delta$, $\alpha 6\beta 3\delta$, $\alpha 4\beta 2\delta$ and $\alpha 6\beta 1\delta$ GABA_A receptors expressed in *Xenopus* oocytes. **b, c** Representative traces for showing membrane currents activated by various GABA concentrations. Traces highlight the high and low affinity components and the plateau response representative of a

bi-phasic concentration response curves at $\alpha 4\beta 3\delta$ and $\alpha 6\beta 2\delta$ GABA_A receptors expressed in *Xenopus* oocytes. **d** Concentration-response curves for GABA at $\alpha 4\beta 1\delta$ (open square, $n = 8$), $\alpha 4\beta 2\delta$ (filled circle, $n = 4$), $\alpha 6\beta 1\delta$ (open circle, $n = 6$), $\alpha 6\beta 3\delta$ (filled diamond, $n = 10$), and compared to $\alpha 1\beta 3\delta$ (open diamond, $n = 4$) GABA_A receptor subtypes. Data are expressed as mean \pm SEM

Given the high sequence identity of the GABA binding site, it is not clear why GABA is so potent at $\alpha 4\beta 3\delta$ GABA_A receptors. One possibility may be that the δ -subunit is not just a substitute for the $\gamma 2$ -subunit, but may contain an additional GABA binding site. Using the $\alpha 4\beta 3\delta$ GABA_A receptor, Karim et al. (2012) found that GABA potency was affected not only by the $\beta 3$ -subunit but, to some extent, by the δ -subunit. Furthermore, mutations in the $\beta 3$ - but not $\alpha 4$ -subunit, known to affect GABA potency on analogous synaptic $\alpha 1\beta 2\gamma 2\text{L}$ GABA_A receptors, had little effect on GABA potency at $\alpha 4\beta 3$ and $\alpha 4\beta 3\delta$ (Karim et al. 2012) and, $\alpha 4\beta 1$ and $\alpha 4\beta 1\delta$ GABA_A receptors (Absalom et al. 2012), inferring that the binding mode of GABA is different for this receptor. In addition, a mutation in loop C of the δ -subunit affected GABA potency possibly

indicating an additional binding site, which may explain its potency on these receptors (Karim et al. 2012).

In conclusion, it is not surprising that differences arise in GABA potency when evaluated using the various expression systems (oocytes versus HEK293 cells for example). This most likely arises come from the amount of cRNA or cDNA that is injected or transfected, but other factors may also play a role (such as phosphorylation state, differences related to the drug application system, the size of the cell, and the presence of endogenous factors in oocytes). In the case of oocytes, the amount of cRNA injected is well controlled. Indeed varying the relative amount of mRNA injected affects what GABA_A receptor subtypes are expressed (Boileau et al. 2002). Boileau et al. (2002) show that mixed populations of binary and ternary receptors are

formed if the ratios are not optimal. In our laboratory, we inject oocytes with excess $\gamma 2$ - or δ -subunit mRNAs (either as 1:1:5, 1:1:10 or 5:1:5 for the $\alpha 4\beta 3\delta$ combination) to avoid contamination by binary receptors that will lower the overall EC_{50} value. In addition, we used Zn^{2+} to ascertain that ternary combinations express as a homogeneous population. However, the slow application speed when using oocytes may desensitize the receptor, increasing the overall EC_{50} value. With the exception of δ -containing $GABA_A$ receptors, the EC_{50} values obtained using oocytes were indeed higher (approx. 4 to 10-fold) compared to those obtained from HEK293 cells (Mortensen et al. 2012), and may reflect the slow application speed.

In summary, differences in GABA potency at the various receptors may reflect that different GABA concentrations are required to activate $GABA_A$ receptors located either synaptically or extrasynaptically. Given that $\alpha 1$ –3-containing receptors located on postsynaptic sites, exposure to high concentrations of GABA is required for activation, while at extrasynaptic sites, receptors composed of $\alpha 4$ –6 require less GABA for activation (Mortensen et al. 2012). Our data reflect this trend and the vast number of human $GABA_A$ receptor subtypes studied will provide a basis for comparing the effect of GABA using the *Xenopus* oocyte system in one laboratory.

Acknowledgments We are grateful to Dr. Paul Whiting (Merck, Sharpe and Dohme Research Laboratories, Harlow, UK) and Dr. Bjarke Ebert (H. Lundbeck A/S Valby, Denmark) for the gift of cDNA for $GABA_A$ subunits. We are very grateful to the Department of Pharmacology, The University of Sydney, for managing and maintaining the *Xenopus laevis* colony. MC acknowledges travel support from the Drug Research Academy, the Faculty of Pharmaceutical Sciences, The University of Copenhagen, Denmark, and the Australian Academy of Sciences. PW acknowledges support from the Alfred Benzon Foundation, Denmark. NK acknowledges The University of Malakand, Pakistan (Faculty Development Programme Scholarship) and the John Lamberton Scholarship. The funding sources solely provided financial support and were not involved in any part of the conduct of the research.

Conflicts of interest The authors have no conflict of interest.

References

- Absalom N, Eghorn LF, Villumsen IS, Karim N, Bay T, Olsen JV, Knudsen GM, Brauner-Osborne H, Frolund B, Clausen RP, Chebib M, Wellendorph P (2012) Alpha4betadelta $GABA(A)$ receptors are high-affinity targets for gamma-hydroxybutyric acid (GHB). *Proc Natl Acad Sci USA* 109(33):13404–13409
- Atack J, Wafford KA, Tye SJ, Cook SM, Sohal B, Pike A, Sur C, Melillo D, Bristow L, Bromidge F, Ragan I, Kerby J, Street L, Carling R, Castro JL, Whiting P, Dawson GR, McKernan RM (2005) The benzodiazepine binding site of $GABA_A$ receptors as a target for the development of novel anxiolytics. *Expert Opin Investig Drugs* 14:601–618
- Barnard EA, Skolnick P, Olsen RW, Mohler H, Sieghart W, Biggio G, Braestrup C, Bateson AN, Langer SZ (1998) International Union of Pharmacology. XV. Subtypes of gamma-aminobutyric acidA receptors: classification on the basis of subunit structure and receptor function. *Pharmacol Rev* 50(2):291–313
- Belelli D, Harrison NL, Maguire J, Macdonald RL, Walker MC, Cope DW (2009) Extrasynaptic $GABA_A$ receptors: form, pharmacology, and function. *J Neurosci* 29(41):12757–12763
- Boileau AJ, Baur R, Sharkey LM, Sigel E, Czajkowski C (2002) The relative amount of cRNA coding for gamma2 subunits affects stimulation by benzodiazepines in $GABA(A)$ receptors expressed in *Xenopus* oocytes. *Neuropharmacology* 43(4):695–700
- Bonnert TP, McKernan RM, Farrar S, Bourdelles BI, Heavens RP, Smith DW, Hewson L, Rigby MR, Sirinathsinghi DJS, Brown N, Wafford KA, Whiting PJ (1999) θ , a Novel γ -aminobutyric acid type A receptor subunit. *Proc Natl Acad Sci* 96(17):9891–9896
- Brandon NJ, Delmas P, Kittler JT, McDonald BJ, Sieghart W, Brown DA, Smart TG, Moss SJ (2000) $GABA_A$ receptor phosphorylation and functional modulation in cortical neurons by a protein kinase C-dependent pathway. *J Biol Chem* 275(49):38856–38862
- Ducic I, Caruncho HJ, Wei Jian Z, Vicini S, Costa E (1995) $GABA$ gating of Cl^- channels in recombinant $GABA_A$ receptors. *J Pharm Exp Ther* 272(1):438–445
- Hadley SH, Amin J (2007) Rat alpha6beta2delta $GABA_A$ receptors exhibit two distinct and separable agonist affinities. *J Physiol* 581(Pt 3):1001–1018
- Hall BJ, Chebib M, Hanrahan JR, Johnston GAR (2005) 6-Methylflavanone, a more efficacious positive allosteric modulator of [gamma]-aminobutyric acid (GABA) action at human recombinant [alpha]2[beta]2[gamma]2L than at [alpha]1[beta]2[gamma]2L and [alpha]1[beta]2 $GABA_A$ receptors expressed in *Xenopus* oocytes. *Eur J Pharmacol* 512(2–3):97–104
- Harpsoe K, Ahning PK, Christensen JK, Jensen ML, Peters D, Balle T (2011) Unraveling the high- and low-sensitivity agonist responses of nicotinic acetylcholine receptors. *J Neurosci* 31(30):10759–10766
- Hosie AM, Dunne EL, Harvey RJ, Smart TG (2003) Zinc-mediated inhibition of $GABA(A)$ receptors: discrete binding sites underlie subtype specificity. *Nat Neurosci* 6(4):362–369
- Hutcheon B, Fritschy JM, Poulter MO (2004) Organization of $GABA$ receptor alpha-subunit clustering in the developing rat neocortex and hippocampus. *Eur J Neurosci* 19(9):2475–2487
- Karim N, Gavande N, Wellendorph P, Johnston GAR, Hanrahan JR, Chebib M (2011) 3-Hydroxy-2'-methoxy-6-methylflavone: a potent anxiolytic with a unique selectivity profile at $GABA(A)$ receptor subtypes. *Biochem Pharmacol* 82(12):1971–1983
- Karim N, Wellendorph P, Absalom N, Bang LH, Jensen ML, Hansen MM, Lee HJ, Johnston GAR, Hanrahan JR, Chebib M (2012) Low nanomolar GABA effects at extrasynaptic alpha4beta1/beta3delta $GABA(A)$ receptor subtypes indicate a different binding mode for GABA at these receptors. *Biochem Pharmacol* 84(4):549–557
- Kaur KH, Baur R, Sigel E (2009) Unanticipated structural and functional properties of delta-subunit-containing $GABA_A$ receptors. *J Biol Chem* 284(12):7889–7896
- Kleingoor C, Wieland HA, Korpi ER, Seeburg PH, Kettenmann H (1993) Current potentiation by diazepam but not GABA sensitivity is determined by a single histidine residue. *NeuroReport* 4(2):187–190
- Knoflach F, Benke D, Wang Y, Scheurer L, Luddens H, Hamilton BJ, Carter DB, Mohler H, Benson JA (1996) Pharmacological modulation of the diazepam-insensitive recombinant γ -aminobutyric acid(A) receptors $\alpha 4\beta 2\gamma 2$ and $\alpha 6\beta 2\gamma 2$. *Mol Pharmacol* 50(5):1253–1261
- Korpi ER, Mihalek RM, Sinkkonen ST, Hauer B, Hevers W, Homanics GE, Sieghart W, Luddens H (2002) Altered receptor

- subtypes in the forebrain of GABA(A) receptor delta subunit-deficient mice: recruitment of gamma 2 subunits. *Neuroscience* 109(4):733–743
- Meera P, Wallner M, Otis TS (2011) Molecular basis for the high THIP/gaboxadol sensitivity of extrasynaptic GABA(A) receptors. *J Neurophysiol* 106(4):2057–2064
- Mortensen M, Patel B, Smart TG (2012) GABA potency at GABA(A) receptors found in synaptic and extrasynaptic zones. *Front Cell Neurosci* 6:1–10
- Nusser Z, Sieghart W, Somogyi P (1998) Segregation of different GABA_A receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *J Neurosci* 18(5):1693–1703
- Olsen RW, Sieghart W (2008) International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacol Rev* 60(3):243–260
- Palma E, Trettel F, Fucile S, Renzi M, Milei R, Eusebi F (2003) Microtransplantation of membranes from cultured cells to *Xenopus* oocytes: a method to study neurotransmitter receptors embedded in native lipids. *Proc Natl Acad Sci USA* 100(5):2896–2900
- Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G (2000) GABA_A receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience* 101(4):815–850
- Rudolph U, Crestani F, Mohler H (2001) GABA_A receptor subtypes: dissecting their pharmacological functions. *Trends Pharmacol Sci* 22:188–194
- Sieghart W (2006) Structure, pharmacology, and function of GABA_A receptor subtypes. *Adv Pharmacol* 54:231–263
- Sigel E, Kaur KH, Luscher BP, Baur R (2009) Use of concatamers to study GABA_A receptor architecture and function: application to delta-subunit-containing receptors and possible pitfalls. *Biochem Soc Trans* 37(Pt 6):1338–1342
- Sinkkonen ST, Hanna MC, Kirkness EF, Korpi ER (2000) GABA(A) receptor ϵ and θ subunits display unusual structural variation between species and are enriched in the rat locus ceruleus. *J Neurosci* 20(10):3588–3595
- Smart TG, Moss SJ, Xie X, Huganir RL (1991) GABA_A receptors are differentially sensitive to zinc: dependence on subunit composition. *Br J Pharmacol* 103(4):1837–1839
- Sperk G, Schwarzer C, Tsunashima K, Fuchs K, Sieghart W (1997) GABA(A) receptor subunits in the rat hippocampus I: immunocytochemical distribution of 13 subunits. *Neuroscience* 80(4):987–1000
- Storustovu SI, Ebert B (2006) Pharmacological characterization of agonists at delta-containing GABA_A receptors: functional selectivity for extrasynaptic receptors is dependent on the absence of gamma2. *J Pharmacol Exp Ther* 316(3):1351–1359
- Wagoner KR, Czajkowski C (2010) Stoichiometry of expressed alpha(4)beta(2)delta gamma-aminobutyric acid type A receptors depends on the ratio of subunit cDNA transfected. *J Biol Chem* 285(19):14187–14194
- Wei W, Zhang N, Peng Z, Houser CR, Mody I (2003) Perisynaptic localization of delta subunit-containing GABA(A) receptors and their activation by GABA spillover in the mouse dentate gyrus. *J Neurosci* 23(33):10650–10661
- Wisden W, Laurie DJ, Monyer H, Seeburg PH (1992) The distribution of 13 GABA_A receptor subunit mRNAs in the rat brain: telencephalon, diencephalon, mesencephalon. *J Neurosci* 12(3):1040–1062
- Wu J, Liu Q, Yu K, Hu J, Kuo YP, Segerberg M, St John PA, Lukas RJ (2006) Roles of nicotinic acetylcholine receptor beta subunits in function of human alpha4-containing nicotinic receptors. *J Physiol* 576(Pt 1):103–118