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COMMENTARY

Pharmacological characterization of a novel cell line expressing human $\alpha_4\beta_3\delta$ $GABA_A$ receptors: commentary on Brown *et al*

*,[1]J.J. Lambert & [1]D. Belelli

[1]Department of Pharmacology and Neuroscience, Neuroscience Institute, Ninewells Hospital and Medical School, Dundee University, Dundee DD1 9SY

We are entering an exciting era of $GABA_A$ receptor research. The cloning of the $GABA_A$ receptor α and β subunits in the late 1980's was followed rapidly by the discovery of, what seemed at the time, to be a bewildering number of novel protein subunits. To date in humans, six α, three β, three γ, three ρ, ε, π, θ and δ subunits have been identified (Barnard *et al*., 1998). Given this subunit repertoire and no restrictions on subunit assembly in forming a functional pentamer, the mammalian central nervous system would be empowered, theoretically, to form thousands of distinct receptor isoforms. However, it soon became evident that these subunits are heterogeneously distributed throughout the central nervous system, and that only certain combinations of subunits are permitted (Moss & Smart, 2001). Nevertheless, estimates suggest something in the order of 20 to 30 important receptor isoforms in the mammalian brain (Whiting *et al*., 1995). The various subtypes of $GABA_A$ receptor exhibit distinct physiological and pharmacological properties, offering the prospect of a new generation of therapeutics for the treatment of conditions such as anxiety, epilepsy and sleep disorders, but hopefully with a reduced propensity for side effects. That such aspirations may be realistic is supported by the results of innovative experiments with so-called 'knock-in' mice, genetically manipulated to express benzodiazepine-insensitive $GABA_A$ receptor isoforms. These studies indicate that distinct $GABA_A$ receptor subtypes mediate the different behavioural effects of diazepam, separating for example the anxiolytic from the sedative effects of this agent (Mohler *et al*., 2002).

By contrast to our knowledge on the impact of α, β, and γ subunits on $GABA_A$ receptor pharmacology, relatively little is known of the properties of δ subunit containing receptors. This has been in part due to difficulties associated with transiently expressing this subunit. However, the paper by Brown *et al*. (2002) now reports on a comparative voltage-clamp study which utilises cell lines engineered to stably express human $\alpha_4\beta_3\delta$ and $\alpha_4\beta_3\gamma_2$ $GABA_A$ receptor subtypes and demonstrates the former to exhibit a unique pharmacology, which coupled with the restricted co-expression of these subunits identifies this subtype of $GABA_A$ receptor as an intriguing target for drug development.

Receptors incorporating α_4 and δ subunits are mainly found in the hippocampus and thalamus (Sur *et al*., 1999; Pirker *et al*., 2000) where co-immunoprecipitation studies suggest that they form ~13% and 7% of total muscimol binding sites respectively in these brain regions. However, arguably the main interest lies not in the relative numbers of the receptor *per se*, but in where it is located. There is an increasing awareness of the importance of extrasynaptic $GABA_A$ receptors in controlling neuronal excitability (Mody, 2001; Brickley *et al*., 2001; Nusser & Mody, 2002). In hippocampal dentate gyrus granule cells for example, it is estimated that the tonic current mediated by extrasynaptic receptors is ~four times greater than that produced by spontaneous inhibitory synaptic currents (Nusser & Mody, 2002). In cerebellar granule cells the δ subunit, in partnership with the α_6 subunit, appears to be exclusively extrasynaptic (Nusser *et al*., 1998), and although such high resolution anatomical detail is currently not available for α_4 and δ subunit containing receptors, a similar extrasynaptic location seems likely (Nusser & Mody, 2002). Certainly, the distinctive interaction of GABA with the $\alpha_4\beta_3\delta$ receptor reported by Brown *et al*. (2002) would support this proposal. Hence, they find this receptor to have a relatively high affinity for GABA and therefore it could be activated by tonic levels of the agonist present in the extracellular space. Furthermore, they demonstrate that the $\alpha_4\beta_3\delta$ receptor is less prone to desensitization, and hence would continue to conduct, in the prolonged presence of the agonist. The interaction of the $GABA_A$ receptor agonist THIP was also distinctive, as it exhibited a maximal response far greater than that elicited by GABA, suggesting that GABA unusually can be regarded as a partial agonist for this receptor.

Clearly the development of pharmacological agents specific for the $\alpha_4\beta_3\delta$ receptor subtype would be of great value in elucidating the role of this receptor isoform in neuronal signaling. The report by Brown *et al*. (2002) offers encouragement for this endeavour. Receptors containing the α_4 subunit, in common with those incorporating the α_6 subunit, are known to be insensitive to classical benzodiazepines such as diazepam and flunitrazepam (Wafford *et al*., 1996). However, if co-expressed with a γ_2 subunit (e.g. $\alpha_4\beta_3\gamma_2$) then bretazenil and Ro15-4513 characteristically enhance GABA-evoked responses, whereas they are inhibited by the 'inverse agonist' DMCM (Brown *et al*., 2002; Wafford *et al*., 1996). By contrast, these agents have no effect on $\alpha_4\beta_3\delta$ receptors (Brown *et al*., 2002), demonstrating that the δ subunit cannot support this characteristic 'benzodiazepine pharmacology'. Etomidate is distinct from other intravenous

*Author for correspondence; E-mail: j.j.lambert@dundee.ac.uk

anaesthetics in that it is highly selective for β_2 and β_3 *c.f.* β_1 subunit containing $GABA_A$ receptors, a specificity dictated by the nature of a single amino acid (Belelli *et al*., 1997). The present study further differentiates etomidate from other general anaesthetics as it is far more effective in enhancing GABA-evoked currents at $\alpha_4\beta_3\delta$ *c.f.*. $\alpha_4\beta_3\gamma_2$ receptors. In a related paper from the same group, they demonstrate that the anxiolytic tracazolate, in common with etomidate, is $\beta_{2/3}$ *c.f.* β_1 selective, but that again the allosteric actions of this drug are additionally favoured by the presence of the δ subunit (Thompson *et al*., 2002).

The interaction of neurosteroids (i.e. steroids synthesized in the central nervous system) with the $\alpha_4\beta_3\delta$ receptor is of particular interest. Certain pregnane steroids are now established as extremely potent, positive allosteric modulators of the $GABA_A$ receptor (Lambert *et al*., 2001; Mellon & Griffin, 2002). Such centrally synthesized steroids are proposed to play a physiological/pathophysiological role to locally influence neuronal excitability by 'fine-tuning' the interaction of GABA with the $GABA_A$ receptor (Lambert *et al*., 2001). The levels of such steroids are not static, but are subject to both cyclical and dynamic changes (e.g. during stress and pregnancy). Given the almost ubiquitous presence of $GABA_A$ receptors throughout the mammalian central nervous system it is important to establish whether such changes in neurosteroid levels would be universally experienced, or would be brain region, or indeed neurone specific. It had previously been reported that incorporation of the δ subunit suppresses neurosteroid sensitivity (Zhu *et al*., 1996). However, Brown *et al*. (2002) now clearly demonstrates a selective, positive interaction of a variety of pregnane steroids with the $\alpha_4\beta_3\delta$ receptor. In particular, the maximal GABA-modulatory effect of the steroids was favoured by the presence of the δ *c.f.* the γ_2 subunit, a conclusion in agreement with a recent study on $\alpha_1\delta$ subunit containing receptors (Wohlfarth *et al*., 2002). Consistent with a selective interaction of the neurosteroids with δ subunit containing receptors, their anaesthetic and anticonvulsant effects are attenuated in a δ subunit knock-out mouse (Mihalek *et al*., 1999). However, the interpretation of these findings may be confounded by compensatory changes in other $GABA_A$ receptor subunits (Tretter *et al*., 2001).

Finally, the expression of both the δ and the α_4 subunit is highly plastic. Following seizures, hippocampal dentate granule cells exhibit considerably increased δ subunit mRNA levels (Brooks-Kayal *et al*., 1998). Withdrawal from neurosteroids, (i.e. mimicking changes that occur during the menstrual cycle and pregnancy), causes an upregulation of the α_4 subunit, with concomitant changes in hippocampal $GABA_A$ receptors (Smith *et al*., 1998).

Therefore in summary, in whole brain $GABA_A$ receptors incorporating both α_4 and δ subunits are in the minority. However, their selective distribution (mainly in the hippocampus and thalamus) and putative extrasynaptic location, coupled with their plasticity, suggests that they may play an important physiological and pathophysiological (e.g. in epilepsy and drug withdrawal) role. Furthermore, the selective pharmacology of this receptor isoform described by Brown *et al*. (2002), should encourage the future development of novel selective ligands, which may have considerable therapeutic potential.

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