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Probing Novel Compound Classes & a New Interacting Protein for the Mammalian $GABA_A$ Receptor

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Abstract

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the vertebrate brain mediating its fast inhibitory action via $GABA_A$ receptors. These receptors are implicated in a number of neurological diseases, making $GABA_A$ receptor ligands interesting as potential therapeutic agents.

The aims of this research project were two-fold: identifying leads for the discovery of new chemical entities that modify $GABA_A$ receptor function. The second aim was to increase the understanding of GABAgeric transmission by studying the pharmacological influence of a new interacting protein for the mammalian $GABA_A$ receptor, GRIF-1.

In the search for novel ligands for $GABA_A$ receptor, the pharmacology of three structurally distinct compound classes was investigated. The first class was the NSAID, Mefenamic acid (MFA) and a group of analogues. Results showed that MFA and a series of analogues selectively modulate $GABA_AR$ at the agonist binding site, but did not interact with either the picrotoxin or the benzodiazepine sites. Indeed the most significant result of this study was the identification of common active conformers of MFA compound and the differentiation of two analogues based on MFA structure, with an improvement in apparent efficacy. The second compound studied was Octyl-β-D-glucoside, a small molecule congener of a natural fungal metabolite, Caloporoside. These studies demonstrated that Octyl-β-D-glucoside is a positive modulator of $GABA_A$ receptor at the channel site demonstrated by its stimulation of specific [^{35}S] TBPS binding. The level of stimulation was similar to that elicited by diazepam and was occluded by GABA. Preliminary structure-activity study showed that the β-glycosidic linkage and chain length are crucial for the positive modulation of [^{35}S] TBPS binding to the $GABA_AR$ by this novel chemical class. The third compound series were essential oils derived from *Melissa officinalis* and *Lavendula angustifolia*. These two oils either singly or in combination have been reported to have a significant benefit in the treatment of agitation in dementia. The purpose of this study was to clarify the sedative and calming mechanisms of these two common essential oils by investigating their effects on the $GABA_AR$ complex. Melissa and Lavender both singly and in combination inhibit [^{35}S] TBPS binding to the channel site of $GABA_AR$. Melissa oil displayed the higher affinity. Melissa oil alone also showed a stimulatory effect on [^{3}H] muscimol binding. Interestingly, a combination effect on the inhibition of [^{3}H] flunitrazepam binding to the $GABA_AR$ has been shown when Lavender and Melissa oils are applied together (50:50), with no effect when applied alone. Neither Melissa nor Lavender oils

demonstrated any effect on the binding of [^{3}H] MK-801 to NMDA receptors, or [^{3}H] nicotine to nicotinic acetylcholine receptors. Furthermore, functional studies have demonstrated that both oils (0.01 mg/ml) applied to rat primary cortical neuron cultures, results in a significant reduction in both inhibitory and excitatory transmission, with a net depressant effect on neurotransmission. These data suggests that the calming/sedative effects of Melissa are mediated by multiple mechanisms in the CNS; the net effect is depressant on the overall neuronal network.

Finally, a pharmacological study was performed on GRIF-1a, a novel $GABA_A$ receptor β2 subunit trafficking protein, to gain further insights into the potential role of this novel protein at the inhibitory synapse. In the present work, evidence was provided that GRIF-1a does not increase α1β2γ2 receptor complex numbers, but appears importantly to stabilise the $GABA_AR$ in a conformation which facilitates binding to both GABA and benzodiazepines. These findings suggest that GRIF-1 protein may be a novel means of modifying the efficacy of synaptic inhibition.

In summary, this thesis provides a clear picture about four novel ways for the modulation of the $GABA_A$ receptor inhibitory transmission.