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Title:
Molecular determinants of GABAA receptor modulation by valerenic acid.

Abstract in Englisch

Valerenic acid (VA) is a positive allosteric modulator of β2/3-containing GABAA receptors and displays anxiolytic and anticonvulsive properties. VA's β2/3-subunit-selectivity is determined by an asparagine residue in β2/3265. However, its binding site on GABAA receptors is still unknown.

In the scope of this thesis, I aimed to identify molecular determinants for potent, efficacious, and β-subunit-dependent modulation by VA: first, I studied the contribution of selected amino acids in the α1- and β3-subunit to VA efficacy and potency. Wild-type α1β3γ2S and mutant GABAA receptors were expressed in Xenopus laevis oocytes and IGABA enhancement by VA was analyzed by means of the two-microelectrode voltage clamp technique. In addition, chemical features essential for potent, efficacious, and β-subunit-dependent modulation were investigated making use of three focused libraries of VA derivatives. Finally, the effect of VA and a previously reported derivative (VA amide; VA-A) was studied on GABAA receptors expressed in human embryonic kidney (HEK) cells and hippocampal neurons. Four transmembrane residues in the β3-subunit (N265, R269, M286, F289) were identified to determine the interaction of VA with GABAA receptors.

These results indicate a VA binding site located at the α-/β+-interface, potentially overlapping with sites for general anaesthetics. Structure-activity relationship (SAR) studies further revealed that modifications of VA's carboxyl function and its two methyl groups in position 3 and 7 profoundly alter efficacy, potency, and β-selectivity. In particular, VA-A modulated synaptic- and extrasynaptic-type GABAA receptors, expressed in both oocytes (α1β2/3γ2S) and HEK cells (α1β2γ2S, α5β3γ2S, α4β3δ, α6β3δ), more potently and/or efficaciously than VA. Both compounds also increased tonic inhibition in hippocampal neurons, probably due to their high activity on α5-containing GABAA receptors.

Taken together, my work suggests a binding pocket for VA at the α-/β+-interface. Increased tonic inhibition by VA and derivatives is reported for the first time. Together with the data obtained from SAR studies, these results may help to develop novel, β-subunit-selective ligands. Future studies are required to investigate a potential contribution of enhanced tonic inhibition by VA and VA derivatives to their in vivo effects.