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Defensio: March 28th 2019

Title of the thesis:

Interaction of GABA-A Receptors with Natural and Protons

Abstract

In this thesis, we focussed on the interaction of various natural products and derivatives as well as protons with different γ-aminobutyric acid type A receptors ($GABA_ARs$) expressed in *Xenopus laevis* oocytes studied by means of two-microelectrode voltage clamp technique (TEVC). The binding pocket of the $\beta_{2/3}$ subunit-selective $GABA_AR$ modulator valerenic acid (VA) was identified and inspired the design of a library of simplified subunit-selective GABAergic ligands with enhanced *in vitro* and *in vivo* activity. Moreover, a zebrafish larvae locomotion assay revealed inhibitory activity of a dichloromethane extract of *Searsia pyroides* which could be attributed to $GABA_AR$ modulation. In the second part, we showed for the first time that homooligomeric $GABA_A$ β_3 receptors can be activated by protons in a concentration-dependent manner suggesting a potential role as "proton receptors".

Recently, VA was identified as $\beta_{2/3}$ subunit-selective $GABA_AR$ modulator. Like etomidate, loreclezole or mefenamic acid, VA's activity strongly depends on the presence of residue $\beta_{2/3}N265$ in the transmembrane domain (TMD). Therefore, utilizing homology modelling VA was docked into a TMD pocket at the β^+/α^- subunit interface on $\alpha_1\beta_3\gamma_{2S}$ receptors suggesting direct interactions between β_3N265/β_1S265 and $\beta_{1/3}R269$ as well as multiple hydrophobic contacts to the lipophilic pocket surface. Mutational analysis showed a complete loss of VA's activity on β_3M286W channels as well as significantly decreased $GABA_AR$ modulation of VA on β_3N265S and β_3F289S receptors indicating that these residues shape the binding pocket of VA near etomidate/propofol/loreclezole binding site(s). A ligand-based pharmacophore model obtained from VA and loreclezole revealed similar chemical features suggesting a common interaction pattern with the binding pocket. The following pharmacophore-guided compound design approach resulted in a library of chemically simplified ligands with compound **12** [(Z)-3-(2,4-dichlorophenyl)but-2-enenitrile] displaying the highest potency (EC_{50}: 13 ± 2 μM) and **18** [(E)-2-Cyano-3-(2,4-dichlorophenyl)but-2-enamide] inducing the highest maximal modulation of GABA-induced chloride currents (Emax: 3114 ± 242%). Furthermore, in hippocampal neurons **12** enhanced phasic and tonic GABAergic inhibition,

and *in vivo* studies revealed significantly more potent protection against pentylenetetrazole (PTZ)-induced seizures compared to VA and LOR.

A dichloromethane extract from *Searsia pyroides* potentiated GABA-induced currents on $\alpha_1\beta_2\gamma_{2S}$ receptors by 172 ± 54% when tested at 100 µg/mL and, in a zebrafish larvae locomotion assay, significantly lowered PTZ-provoked locomotion at 4 µg/mL. Using HPLC-based activity profiling and the zebrafish assay, active compounds **1-3** and structurally related compounds **4-6** were tracked and isolated. Compounds **1-3** were identified as derivatives of anacardic acid and showed decrease of PTZ-provoked locomotion in the zebrafish larvae model in a concentration-dependent manner, while **4-6** were inactive. Compounds **1-3** enhanced I_{GABA} on $\alpha_1\beta_2\gamma_{2S}$ receptors concentration-dependently, while **4-6** only showed negligible effects, confirming results from the zebrafish assay.

Based on the pharmacological characterization of a functional prokaryotic/eukaryotic pentameric ligand-gated ion channel chimera we showed for the first time that homooligomeric rat $GABA_A$ β_1 and β_3 receptors can be gated upon proton-application in a concentration-dependent manner (pH_{50}: 5.5 ± 0.2 and 6.1 ± 0.1, respectively). Structural and experimental studies identified H267 as an essential proton-sensing residue and Molecular Dynamics simulations suggested H267-E270-interaction as a potential critical mechanism underlying proton-induced gating of homomeric β_3 receptors.

We conclude that the natural compounds studied in this thesis constitute promising scaffolds for the development of novel $GABA_AR$ modulators with anticonvulsant-like activity. Furthermore, we demonstrated so far unknown proton-induced activity of homooligomeric $GABA_ARs$ suggesting a role as proton-receptors responding to pH alteration in the central nervous system under certain conditions.