Molecular Neurobiology Synaptic Plasticity

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From the GABA-B receptor proteome to brain functions and therapeutic concepts

My laboratory studies how the molecular composition of GABA-B receptors (GBRs), the G protein-coupled receptors for the neurotransmitter GABA, influences neuronal activity. Because GBRs are implicated in the pathophysiology of neurological and psychiatric disorders, we also aim at targeting molecularly defined GBR signaling complexes for therapy. In collaboration with Prof. B. Fakler (University of Freiburg, Germany), we identifed 30 GBR-associated proteins (Pin & Bettler, Nature 540, 2016; Bettler & Fakler, Curr. Opin. Neurobiol. 45, 2017). We have mapped the interactions of several of these proteins with each other and with the GBR subunits GB1 and GB2 (Fig. 1). We found that GBR components associate in a modular fashion into a variety of functionally distinct multi-protein complexes. We analyzed several GBR-associated proteins for their effects on receptor signaling, neuronal excitability, brain network activity and behavior. Auxiliary KCTD proteins, for example, regulate the kinetics of GBR-induced currents, explaining kinetic discrepancies between currents observed in different neurons (Fritzius *et al.,* J. Neurosci. 37, 2017). KCTD proteins influence both strength and frequency of thalamic spindle oscillations, showing that kinetic effects of the KCTDs on GBR signaling regulate network activity (Ulrich *et al.,* Neuropharmacol. 136, 2018). Accordingly, lack of KCTD16 in mice also influences behavioral responses (Cathomas *et al.,* Behav. Brain Res. 317, 2017).

Amyloid precursor protein (APP), adherens junction-associated protein 1 (AJAP1) and PILRα-associated neural protein (PIANP) form three mutually exclusive GBR complexes by binding to the N-terminal sushi domain of GB1a (Schwenk *et al.,* Nat. Neurosci. 19, 2016). Because this sushi domain mediates axonal localization, we tested whether axonal trafficking of GBRs is impaired in APP-/-, AJAP1-/- or PIANP-/- mice (Dinamarca *et al.,* Nat. Commun. 10, 2019). Selectively APP-/- mice exhibited a decrease in axonal GBRs (Fig. 2A) and a consequent deficit in GBR-mediated inhibition of neurotransmitter release. Trafficking of APP/GBR complexes in axons was visualized using time-lapse imaging (Fig. 2B-D). APP associates with JIP3 and CSTN3 proteins (Fig. 1) of the axonal trafficking machinery. Complex formation with GBRs stabilizes APP at the cell surface and reduces proteolysis of APP to Aβ, a component of senile plaques in Alzheimer's disease. These findings establish a link between APP/GBR complex formation, axonal trafficking of GBRs and Aβ production.

The SHRM4 protein, which is genetically associated with intellectual disability and epilepsy, controls GBR cell surface expression. Knockdown of Shrm4 in rodents impairs GBR activity, induces anxiety-like behaviors and increases susceptibility to seizures (Zapata *et al.,* Nat. Commun. 8, 2017). Collaborative work further showed that GBRs shape the auditory map (Vickers *et al.,* Neuron 99, 2018), evoke distinct responses in astrocytes (Mariotti *et al.,* Nat. Commun. 9, 2018) and regulate cocaine-induced behaviors (Edwards *et al.,* Nat. Neurosci 20, 2017).

Drug Discovery

Clinical use of GBR drugs is limited to agonists and the treatment of narcolepsy, spasticity and alcohol use disorders. One reason for the limited use of GBR agonists is that global GBR activation results in unwanted side effects. The recognition that GBRs form a variety of multi-protein complexes provides opportunities for targeting cell-type specific functions and individual signaling pathways (Rosenbaum *et al.,* Nat Rev Drug Discov (2020). https://doi.org/10.1038/s41573-020-0086-4). Targeting molecularly defined receptor complexes should reduce side effects and enable new therapeutic applications. In collaboration with the medicinal chemistry group of K. Strømgaard (Pharmaceutical University, Copenhagen), we developed

peptide-based inhibitors of the GBR/KCTD interaction (Sereikaite *et al.,* J. Med. Chem. 62, 2019). These inhibitors are expected to exhibit anxiolytic properties by preventing GBR desensitization. We are currently also developing peptides interfering with defined presynaptic GBR complexes to facilitate glutamate release, particularly for the treatment of cognitive dysfunctions.

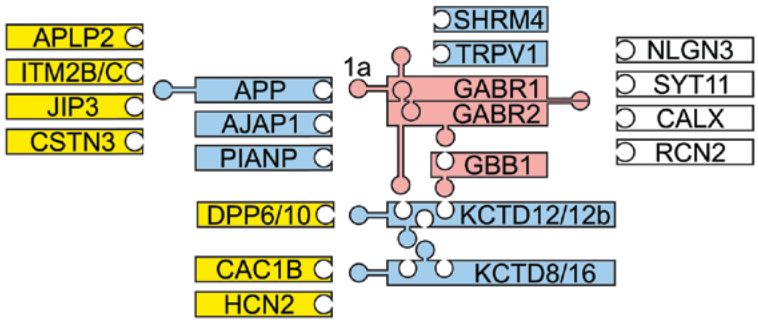

Fig. 1: Structural organization of GBR complexes. GB1 (GABR1) and GB2 (GABR2) receptor subunits constitute fully functional heterodimeric receptors that bind to the β subunit (GBB1) of the G protein (red). Modular association of these receptors with additional components generates multi-protein complexes of varying composition and signaling properties. Primary (blue) and secondary interactors (yellow) as well as receptor components with unknown interaction sites (white) are depicted. Pentamers of auxiliary KCTD proteins bind to GB2 and GBB1 to regulate receptor kinetics. APP, AJAP1 and PIANP bind to the N-terminal sushi domain of GB1a. APP binds to JIP3 and CSTN3, which link the APP/GBR complex to the axonal trafficking motor. APLP2 and ITM2B/C assemble via APP with GB1a. TRPV1 channels bind to GB1a while Cav2.2 (CAC1B) and HCN2 channels bind to KCTD8/16. NLGN3, Neuroligin 3; SYT11, Synaptotagmin 11; CALX, Calnexin; RCN2, Reticulocalbin 2. Adapted from Fritzius & Bettler, Basic Clin. Pharmacol. Toxicol. 126, 2020.

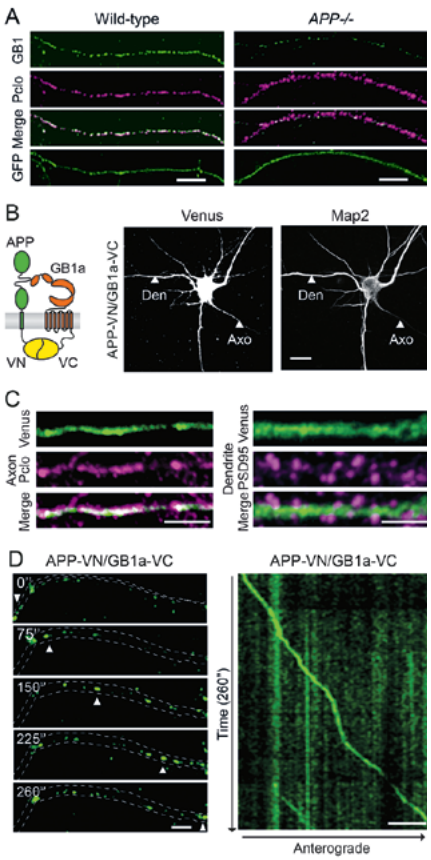

Fig. 2: APP associates with GB1a and mediates axonal trafficking of GBRs. **(A)** Endogenous GB1 protein is reduced by 75% in the axons of APP-/- hippocampal neurons. Neurons were immunostained for GB1 (green) and the presynaptic marker piccolo (Pclo, magenta). GFP served as a volume marker. Merged images show GB1 and piccolo co-localization. Scale bar 5 µm. **(B)** Scheme depicting bimolecular fluorescence complementation (BiFC) using the split Venus fusion-proteins APP-VN and GB1a-VC. Association of APP-VN with GB1a-VC reconstitutes Venus fluorescence. Representative confocal images show hippocampal neurons expressing APP-VN together with GB1a-VC. BiFC is observed in axons (Axo) and dendrites (Den). Microtubule-associated protein Map2 identifies dendrites. Scale bar 10 µm. **(C)** Higher magnification of axons and dendrites expressing APP-VN and GB1a-VC. The BiFC complex (Venus) partly co-localizes with piccolo (magenta) and is also present along dendritic shafts but excluded from spines, identified by PSD-95 (magenta). Scale bar 5 µm. **(D)** Time-lapse images of a well-separated APP-VN/GB1a-VC complex (arrowheads) trafficking anterogradely in the axon (acquisition time in seconds). A kymograph shows the entire time-lapse recording (right). Scale bars 25 µm.

Connection to Clinical Practice

Prof. Murim Choi, Prof. Cyrill Géraud
Seoul National University College of Medicine, Seoul, Republic of Korea (MC), Medical Faculty, University Heidelberg (CG)

GABBR2 and PIANP mutations in neurodevelopmental disorders

Dominant de-novo mutations in the GB2 subunit gene GABBR2 were identified in pediatric Rett (RTT) and epileptic encephalopathy (EE) patients (e. g. Lopes *et al.,* J Med Genet 53, 2016; Yoo *et al.,* Ann Neurol 82, 2017; Vuillaume *et al.,* Ann Neurol 83, 2018). Characterization of RTT and EE mutations revealed that increased constitutive receptor activity reduces the efficacy of GABA. We hypothesize that a reduced efficacy of synaptically released GABA tips the excitation/inhibition balance in the brain towards more excitation. Our collaborator, Prof. M. Choi, has generated mice with inducible RTT and EE mutations in GABBR2. We will study the mechanism of pathogenesis and address how GABBR2 mutations affect synaptic GBR responses to identify possible therapies. A homozygous nonsense mutation in the gene for the GBR-associated protein PIANP leads to global developmental delay and intellectual disability. In collaboration with Prof. C. Géraud, we showed that PIANP-/- mice exhibit autism spectrum disorder-like phenotypes (Winkler *et al.,* Mol. Psych. 2019). Similar to APP-/- mice, PIANP-/- mice exhibit a deficit in GBR-mediated inhibition of glutamate release, supporting that saturation of synaptic plasticity and excitotoxicity contribute to disease pathology.

Selected Publications

Dinamarca MC, Raveh A, Schneider A, Rem PD, Stawarski M, Früh S, Fritzius T, Lalanne T, Turecek R, Choo M *et al.* (2019). Complex formation of APP with $GABA_B$ receptors links axonal trafficking to amyloidogenic processing. Nature Commun. 10 (1), 1331.

Vickers ED, Clark C, Osypenko D, Fratzl A, Kochubey O, Bettler B, and Schneggenbruger R (2018). Parvalbumin-interneuron output synapses show spike-timing dependent plasticity that contributes to auditory map remodeling. Neuron 99(4), 720–735.

Mariotti L, Losi G, Gomez-Gonzalo M, Melone M, Chiavegato A, Lia A, Sessolo M, Bovetti S, Forli A, Zonta M *et al.* (2018). Interneuron-specific signalling unveils unique somatostatin-mediated properties of astrocytes in adult neocortex. Nature Commun. 9(1), 8.2.

Edwards NJ, Zhang S, McDevitt RA, Tejeda HA, Pignatelli M, Bass CE, Bettler B, Morales M, and Bonci A (2017). Circuit specificity in the inhibitory architecture of the VTA regulates behavioral reponses to cocaine. Nature Neurosci. 20(3), 438–448.

Fritzius T, Turecek R, Seddik R, Kobayashi H, Tiao J, Rem PD, Metz M, Kralikova M, Bouvier M, Gassmann M *et al.* (2017). KCTD hetero-oligomers confer unique kinetic properties on hippocampal $GABA_B$ receptor-induced K^+-currents. J. Neurosci. 37(5), 1162–1175.