Abstract Book

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P1. Origin of novel allosteric modulators of cannabinoid receptors: Peptide endocannabinoids

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The cannabinoid receptors (CB) can be modulated by endogenous peptides named peptide endocannabinoids (pepcans) which were elucidated thanks to hemopressin [1,2]. The whole family of pepcans consists of N-terminal extended versions of pepcan-12 (RVD hemopressin), the most biological active peptide with a dual function (CB1 negative allosteric modulator and CB2 positive allosteric modulator) [1,3]. Pepcan-23 is the longest found pepcan and shows no biological activity at the CB receptors.
Localization experiments with ELISA and LC-MS/MS showed pepcans specifically present in noradrenergic neurons in the locus coeruleus of the CNS as well as in chromaffin cells of the adrenal medulla [4]. This was the first indication of a connection between pepcans and the sympathetic nervous system.
The adrenal glands are assumed to be a site of production or storage of pepcan-12 since analytical quantification experiments indicated high amounts of pepcan-12 in mouse adrenal glands but a lack of pepcan-23. To address this hypothesis, quantification studies with LC-MS/MS on adrenalectomized mice were performed, showing an involvement of the adrenal glands in constitutive pepcan production.
Pepcans are derived from the α-hemoglobin gene [1,2] however, the coding sequence between the α-hemoglobin 1 and 2 is identical. Our recent qPCR data suggests that the HBA-A2 gene has a prominent role in generating pepcans within the adrenal glands.
Next to pepcan-12, pepcan-23 is found in high amounts in mice tissues but it does not show a biological activity at the CB receptors. Based on quantification experiments in mice tissues as well as *ex vivo* and *in vivo* experiment, pepcan-23 is the potential precursor of pepcan-12. It has an increased stability in serum, plasma and whole blood compared to pepcan-12 and shows metal binding properties.

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P2. The role of cell-specific 2-AG production in anxiety- and depression-like behavior

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The endogenous cannabinoid system (ECS) is an important neuromodulatory system, involved in a wide range of physiological functions. Behavioural studies have shown that emotional responses are affected by the ECS and that disruption of endocannabinoid signaling contributes to the development of affective disorders. We have recently shown this using knockout mice lacking diacylglycerol lipase alpha (DAGL α), the main producing enzyme of 2-arachidonoylglycerol (2-AG). 2-AG levels in these mice were reduced by more than 80%. In addition, anandamide (AEA) and arachidonic acid (AA) levels were also decreased. Constitutive D AGLα knockout mice displayed a number of behavioral changes, which are indicators of an anxiety-like phenotype (open-field, zero-maze, hypophagia test). Depression-related behaviors were also affected by DAGLα deletion (sucrose preference, forced swim test). In addition, they showed a reduced adult hippocampal neurogenesis, which is also frequently observed in animal models of depression.
Besides neurons, astrocytes and microglia also have the ability to produce endocannabinoids in the brain. However it is currently completely unclear to what extend these endocannabinoids modulate behavior.
Our aim is to clarify the role of 2-AG production by neurons, astrocytes and microglia in regulation of anxiety- and depression-related animal behaviors.
Therefore we are testing conditional DAGLα knockout mice with a specific DAGLa knockout in neurons, astrocytes and microglia in different behavioral tests and for altered adult neurogenesis.
Until now we have found that the loss of 2-AG production by astrocytes seems to be involved in the development of depression-like behavior, also represented in reduced adult neurogenesis.

P3. Distorted endocannabinoid signaling in SGIP1 knock-out mice correlates with altered modulation of anxiety, fear, stress and pain processing

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The endogenous cannabinoid system modulates synaptic transmission and plasticity, and affects important physiological processes such as pain sensation, memory and other responses to the environment, including affection and mood. SH3 domain GRB2 like endophilin interacting protein 1 (SGIP1) interacts with cannabinoid receptor 1 (CB1R) in mammalian brain, and modifies its signaling properties when expressed in heterologous system. We developed SGIP1 knock out mice that show signs of modified exploratory phenotype, and reduced reaction on stressful environment. The de letion of SGIP1 also led to increased threshold responses to pain stimuli. Moreover, the SGIP1KO mice have specific phenotype signs implying on distorted endocannabinoid signaling, including the impact of Δ9 tetrahydrocannabinol (Δ9 THC) in several behavioral paradigms. Depolarization-induced suppression of excitation (DSE), a form of cannabinoid-mediated plasticity, is diminished in neurons lacking SGIP1. We propose, that this change in synaptic plasticity may lead to the phenotype observed in the SGIP1 KO mice.

P4. GRK3 and SGIP1 regulate CB1 receptor signaling

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Cannabinoid receptor 1 (CB1R) is a target for endocannabinoids and phytocannabinoids, such as THC. Besides its canonical regulation by G-protein coupled receptor kinases (GRKs) common to most GPCRs, CB1R signaling is also modulated by the protein SGIP1. GRK3-mediated phosphorylation of the intracellular carboxyterminus of CB1R leads to its desensitization.

How different post-translational modifications of CB1R affects its interaction with SGIP1 is unknown. The aim of this study is to describe how CB1R phosphorylation modifies the modulatory effects of SGIP1 protein on CB1R signaling. We prepared a set of C-terminus CB1R mutants, defective in phosphorylation, or mimicking phosphorylation of relevant Serine and Threonine residues. These mutants were transiently expressed in HEK293 cells to study their signaling properties in the presence, or absence of SGIP1.

We have found that SGIP1 enhanced the association of GRK3 with activated CB1R and those mutated versions that still interacted with GRK3. Likewise, β-arrestin2 binding was affected by the phosphorylation pattern of the CB1R C-tail, and when present, its association was enhanced by SGIP1. The receptor mutants with disturbed β-arrestin2 association still had preserved portion of the ERK1/2 signaling, indicating the involvement of G proteins was retained.

In summary, our data shows that phosphorylation of the CB1R carboxyterminal at residues S426 and S430, and those within the extreme C-tail, play important roles in regulating CB1R signalling in a pathway specific manner. The presence of SGIP1 further tunes this regulation of CB1R activity, as well as the receptor association with regulatory and signaling molecules.

P5. The selective endocannabinoid reuptake inhibitor WOBE437 attenuates disease progression in a mouse model of Multiple Sclerosis

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Multiple sclerosis (MS) is characterized by neurodegeneration associated with demyelination and excitotoxicity. Cannabis has been used for decades to mitigate MS-associated symptoms and preclinical evidence support the role of the endocannabinoid system (ECS) as a key target [1-2]. Endocannabinoid (EC) increase has shown positive results in mouse models of MS; however, they might have limitations in clinical settings for chronic use [3]. Here we analyze the therapeutic potential of WOBE437, a potent selective EC reuptake inhibitor (SERI) which is orally bioavailable and it has shown anxiolytic, analgesic and anti-inflammatory effects in different animal models, including mitigation of chronic inflammation through a polypharmacological mechanism [4-5]. Chronic-progressive experimental autoimmune encephalomyelitis was induced in C57BL/6 mice as a model of MS. Daily treatment (i.p.) with vehicle or WOBE437 10 mg/kg started individually at the disease onset and lasted 20 days. Our results indicate that WOBE437 significantly reduces the disease severity and favors an earlier recovery, mediated mainly through a CB2 receptor-dependent mechanism. CB1 receptor itself seemed to play an important role for disease severity, as its blockage was detrimental for the mice. After the chronic treatment with WOBE437 (10 mg/kg, i.p.), an increase in the CNS levels of AEA and 2-AG was observed without inducing desensitization of brain CBs receptors. Ongoing experiments aim to evaluate the immune cell activation and infiltration during the peak of the disease. Our preliminary results show that SERIs have the potential to be a new strategy for slowing MS progression. Although, it remains unclear whether this mouse model adequately simulates the disease pathophysiology in humans, it is noteworthy that many of the drugs that are in current or imminent use in MS have been developed, tested or validated on the basis of EAE studies [6-7].

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P6. Concept poster: Evaluation of selective endocannabinoid reuptake inhibitors in a mouse model of post-traumatic stress disorder

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Post-traumatic stress disorder (PTSD) is a severe chronic stress and anxiety disorder that develops after the exposure to actual or threatened death, serious injury, sexual violence or other traumatic events. PTSD treatments combine psychotherapy with pharmacotherapy; however, poor remission is observed [1]. The endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are crucially involved in the main signaling circuits that modulate fear, anxiety and stress response and there is an association among dysregulation in endocannabinoid signaling and the physiopathology of PTSD [2,3]. Increment in either AEA or 2-AG levels, through inhibition of its degradation, shows improvements in memory extinction and anxiety-like behavior [4,5]. Recently, we reported that the first selective endocannabinoid reuptake inhibitor (SERI) and orally bioavailable WOBE437 increases both AEA and 2-AG levels *in vitro* and *in vivo*, along with anti-inflammatory, analgesic, and anxiolytic effects in mice [6,7]. After repeated administrations, WOBE437 increases corticosterone levels in healthy mice [6]. Interestingly, patients suffering from PTSD and other stress related disorders (e.g. burnout) are characterized by a pathological impairment of the HPA axis which leads to significantly lower cortisol levels [8,9]. Looking towards a possible clinical translation, we have developed a second generation of SERIs based on a drug-like 2-immino-5-ene-thiazolidin-4-one scaffold. The lead compounds of this class showed an optimal safety profile, good oral bioavailability and similar pharmacological effects *in vivo* compare to WOBE437. Our aim is to evaluate the potential effect of SERIs of first and second generation in a mouse model of PTSD. Single and repeated administration(s) of SERIs will be tested in conditioned and sensitized fear paradigms [10]. Anxiety and freezing behavior will be analyzed along with the quantification of endocannabinoid and corticosterone levels. The involvement of CB1 receptors will be addressed by using pharmacological tools or genetic approaches.

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P7. Design, synthesis and characterization of a novel clickable COX-2 selective probe

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Cyclooxygenase, known as prostaglandin-endoperoxide synthase, is a family of isoenzymes that are responsible for the formation of prostanoids, including thromboxanes, prostaglandins and prostacyclins, from arachidonic acid.
One of the isoforms, the COX-2, highly expressed during inflammatory processes, is also known for playing an important role in the metabolism of the endocannabinoids AEA and 2-AG. From the structural point of view, COX-2 is a sequence homodimer that functions as a conformational heterodimer. One can distinguish between an allosteric monomer and a catalytic monomer. Each cyclooxygenase monomer possesses a peroxidase site POX and a cyclooxygenase site. Both monomers have a binding site for HEME, the cofactor of the enzyme, which is fundamental for its activity and defines the active site where the substrate is oxygenated.
With the idea of developing a fluorescence tool to investigate the dynamic regulation of this enzyme, we synthesized a new chemical probe that labels COX-2. The probe is able to bind covalently and selectively a new binding site located in the HEME binding pocket, the mechanism of which we aim to elucidate by proteomics. This new chemical tool might allow a kinetic assessment of COX-2 protein expression in life cells, but it could also pave the way for exploring a different way to modulate the activity of the COX-2 enzyme, allowing a molecular investigation of the importance of HEME binding. Moreover, this probe might be useful to study the substrate-selective inhibitors that selectively inhibit endocannabinoid metabolism by COX-2.

P8. PHARMACOLOGICAL CHARACTERIZATION OF PEPTIDIC CANNABINOID ALLOSTERIC LIGANDS

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The endocannabinoid system has important physiological functions in the central nervous system and in periphery. Therefore, compounds interacting with the endocannabinoid signaling may have high therapeutic potential in the treatment of inflammatory, neuropathic and other neurological diseases. It has been shown that the CB1 receptor contains allosteric binding sites, which are topologically distinct from that of the orthosteric site. Pepcan-12 (RVD-hemopressin, RVD-Hpα), the most abundant peptide endocannabinoid among the alpha hemoglobin derived hemopressin-like peptides that were identified both in the CNS and in the periphery can interact with the CB1 allosteric binding site(s). Pepcan-12 has been recently described as the first endogenous negative allosteric modulator of cannabinoid CB1 receptors acting at the same time as a potent CB2 receptor positive allosteric modulator. Modulation of CB1/CB2 receptors by this peptide leads modulates signaling pathways in a distinct manner, making Pepcan-12 and its derivatives excellent lead compounds for the development of peptidic therapeutic agents with potentially reduced side effects.
In this study, we report on the synthesis and radiolabeling of Pepcan-12 and the direct in vitro pharmacological characterization of the resulting radioligand [3H]Pepcan-12 in WT and CB1-KO mouse brain membrane homogenates. Truncated hemopressins and synthetic allosteric modulators were investigated in competition binding studies using [3H]Pepcan-12.