Christian Njoo
Dr. Sc.hum.

Proteomics-based identification of interaction partners of cannabinoid receptor 1 and analyses of their roles in structural and functional plasticity of neurons

Promotionsfach: Pharmakologie
Doktormütter: Prof. Dr. Rohini Kuner

Cannabinoids have been use since ancient times as analgesics. Although the cannabinoid receptor 1 (CB1) plays a pivotal role in mediating cannabinoid-induced analgesia, the underlying molecular signaling mechanisms are still poorly understood. This dissertation focuses on a proteomics-based analysis of the CB1 receptor complex in order to unravel novel signaling mechanisms that may play important role(s) in the downstream modulation of neuronal structure and function by cannabinoids. Our mass spectrometry-based analysis revealed a large number of proteins that were specifically co-immunoprecipitated with CB1, amongst which the WAVE1-complex was the most prominent and present in almost its entirety. Through detailed *ex vivo* analyses we showed that this association is specific and functional, thus, providing strong evidence for a novel role of the WAVE1 complex in CB1 signaling. Based on these results, we undertook further *in vivo* studies to investigate the functional role of this interaction in modulation of nociception and inflammatory pain.

Thorough analyses of the CB1-WAVE1 interaction in developing cortical neurons in vitro revealed that CB1-WAVE1 interaction has functional effects on the regulation of growth cone morphology. Further analyses also demonstrated roles of Rac1 and G_i in mediating these functional effects. We also observed that modulation of CB1 activity by an agonist or inverse agonist led to reciprocal changes in the phosphorylation state of WAVE1 thereby regulating its activation towards ARP2/3 and actin nucleation. These experiments also revealed CDK5 as another interacting partner in the CB1-WAVE1 signaling cascade. Since WAVE1 is also known as a nucleation promoting factor protein, we then analyzed the potential role(s) of CB1 in the direct regulation of actin. Indeed, in HeLa cells that heterologously express CB1, we observed that ACEA-induced treatment led to an increased actin regulation at the cell membrane, which we further reconfirmed by demonstrating the translocation of MAL, a downstream target of actin signaling, into the nucleus.

In our *in vivo* study, intrathecal treatment of ACEA led to profound analgesia in mice with CFA-induced inflammation, but only if the treatment was carried out prior to the CFA injection. Moreover, using siRNA-mediated WAVE1 knock down we observed that this analgesic effect of ACEA in the CFA-treated mice was abolished, which suggests a role of the WAVE1 in CB1-induced analgesia in this pathological condition. Interestingly we also observed that intrathecal ACEA-treatment in naïve mice led to mechanical hypersensitivity. This suggests that CB1 modulation is bi-directional and state-dependent. We further investigated the morphology of second order sensory neurons in the spinal dorsal horn of these mice and observed that CFA-induced inflammation led to an increase in the density of dendritic spines in these neurons and that spinal ACEA application negated this effect. Accordingly, ACEA-induced mechanical hypersensitivity in naïve mice also led to an increase in the dendritic spine density in these neurons, further supporting the hypothesis of a state-dependent bi-directional modulation by CB1. WAVE1 downregulation also inhibited the effect of ACEA in the regulation of dendritic spines in naïve mice, but not in inflammatory pain states. We observed that the downregulation of WAVE1 itself abolished the effect in inflammation-induced increase in dendritic spine density in spinal neurons.

Taken together, our study identified WAVE1 as a novel interacting partner of CB1 and elucidated the signaling mechanism underlying this interaction. Importantly, our *in vivo* analyses clearly indicate that WAVE1 plays a functional role in CB1-induced modulation of nociception. Moreover, our *in vitro* and *in vivo* results showed that the downstream signaling of this interaction involved the regulation of actin filaments, which is a key mechanism of any structural changes observed at supraspinal sites. Defects in this mechanism have been shown to underlie various neuropathological conditions such as Alzheimer's disease and drug addiction, both which CB1 has been previously indicated to play a major role as well. Therefore, it would be very interesting to investigate the role(s) of CB1-WAVE1 interaction in different pathological conditions in future studies, especially those that has been shown to involve actin regulation, which will provide more insights into the development of novel drugs.