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PERIPHERAL FUNCTIONS OF THE ENDOCANNABINOID SYSTEM: PATHOLOGY AND PHYSIOLOGY

By Andreas Zimmer, PhD
Institute of Molecular Psychiatry, University of Bonn, Bonn, Germany
neuro@uni-bonn.de

Introduction

Although the endocannabinoid system (ECS) is best known for its important neuromodulatory role, it has become apparent in recent years that the physiology of almost every organ is affected by the ECS. This is especially true under pathological conditions, in which an up-regulation of the expression of cannabinoid receptors and increased endocannabinoid production can often be observed. The ECS in peripheral tissues thus seems to constitute an important stress-response system that becomes activated in situations that challenge homeostasis.

Key Points

- Peripheral CB1 and CB2 receptors contribute to many pathological conditions.
- CB2 receptor signalling has bee implicated in the modulation of immune functions and bone remodelling.
- CB2 receptor deficient mice have a low bone mass phenotype that is very reminiscent of human postmenopausal osteoporosis.
- CB2 receptor signalling contributes to the containment of neuropathic pain states by modulating IFN-γ responses.
- CB2 receptor agonists show efficacy in animal models of osteoporosis and neuropathic pain.

State of the Art

Transgenic and knockout mouse models, in combination with high affinity small molecules, are very powerful tools in the analysis of protein functions. We have generated mutant mouse strains deficient in CB1 and CB2 receptors [1, 2], as well as CB1/CB2 double knockout mice [3, 4]. The lack of each of these receptors has severe deleterious consequences on animal physiology. While most of the initial studies on CB1 receptor knockout animals have focused on central nervous system phenotypes, it has recently been demonstrated that CB1 signalling affects numerous peripheral organs and tissues including bone, liver, skin, the immune system, endothelial cells, adipose tissue, embryo development and implantation, etc. [4-8]. Likewise, CB2 receptor signalling modulates immune cell functions and thus affects many organs and different pathologies, such as atherosclerosis, liver fibrosis, osteoporosis, and neuropathic pain [9, 10].

Neuropathic pain conditions resulting from nerve injury are difficult to treat even with potent analgesic compounds, but recent pharmacological studies indicate that CB2 selective agonists show analgesic efficacy in neuropathic pain conditions. Studies in CB2 knockout mice exposed to sciatic

nerve injury have demonstrated that CB2 receptors contribute to the local containment of neuropathic pain by modulating glial activation in response to nerve injury [11]. In the absence of CB2 signalling, neuropathic pain spreads beyond the site of nerve injury. The underlying molecular mechanisms were identified by expression profiling studies that showed an enhanced interferon-γ (IFN-γ) response in the absence of CB2 signalling [12]. IFN-γ is produced by neurons and astrocytes in the spinal cord segment ipsilateral to the nerve injury in wild-type animals and also in the contralateral region in CB2 deficient mice, thus matching the pattern of nociceptive hypersensitivity in these animals. The most direct support for a functional involvement of IFN-γ as a mediator of CB2 signalling was obtained with a double knockout mouse strain deficient in CB2 receptors and IFN-γ, which no longer showed the contralateral hyperalgesia observed in CB2 knockouts.

The effect of CB2 signalling in neuropathic pain exemplifies the immunomodulatory role of this system under pathological conditions. However, there is still relatively little information about the significance of CB2-mediated effects in normal physiology. In fact, the only obvious defects in CB2 deficient animals, in the absence of pathogenic challenges, have been observed in the skeletal system [13]. During their first 2-3 months of life, CB2-deficient mice accrue a normal peak trabecular bone mass but subsequently show markedly enhanced age-related bone loss. At one year of age, their trabecular bone volume density is approximately 50% lower than that of wild type control animals. Young mice and other vertebrates alike undergo a rapid skeletal growth phase accompanied by accrual of peak bone mass, which remains constant in adult animals until the beginning of age-related bone loss [9]. These changes are referred to as bone remodelling and involve a continuous process of resorption by osteoclasts and formation of new mineralized matrix by osteoblasts. Imbalanced bone remodelling leads to bone mass accrual or bone loss. CB2-deficient mice show an increase in bone resorption and formation, with a net negative balance [13, 14]. This phenotype is reminiscent of human postmenopausal osteoporosis and suggests that CB2 is associated with maintaining bone remodelling at balance. Importantly, CB2-selective agonists were shown to prevent bone loss after ovariectomy [13]. Thus, CB2 is an attractive new target for the pharmacotherapy of osteoporosis.

To determine if CB2 receptors also contribute to the regulation of bone mass in humans, we therefore studied polymorphisms in the human CNR2 locus, encoding the CB2 receptor, in a case-control sample of osteoporotic patients [15]. The CNR2 locus is located on chromosome 1p36. Although this region and its mouse ortholog on chromosome 4 have been previously linked to bone mineral density and osteoporosis in several independent studies [16], none considered CNR2 as a potential candidate gene. We analyzed 26 SNPs spanning approximately 300 kb around the CNR2 locus and found a significant association with the disease phenotype with several SNPs. The most significant P-values were observed with SNPs located within the CB2 coding region. We therefore sequenced the CB2 coding exon in all 388 patients and controls and identified two missense variants, Gln63Arg and His316Tyr, with the Arg63 variant being more common in the osteoporotic patients than in the healthy controls. Our findings were recently confirmed in an independent study using a large cohort of Japanese women and men [17]. Together, these studies strongly suggest that a common variant of the CB2 receptor contributes to the etiology of osteoporosis in humans.

Priority for Future Studies

One important focus in the analysis of human CB2 signalling will be to investigate potential differences in the biochemical properties of the two CB2 receptor variants. These studies have to consider intracellular signalling cascades, receptor sorting and desensitization, receptor heterodimerization, etc. Many of these studies can probably be done in heterologous expression system or in cells isolated from human probands. However, it may ultimately be necessary to generate mice carrying the human receptor isoforms in order to fully elucidate the functional relevance of the variants in the context of pathology. Considering the widespread effects of the ECS on different pathologies, it will also be important to study the association of human receptor polymorphisms with other relevant diseases, such as liver fibrosis.

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