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Anti-inflammatory cannabinoids in diet

Towards a better understanding of CB_2 receptor action?

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Abstract

The endocannabinoid system is an ancient lipid signaling network which in mammals modulates neuronal functions, inflammatory processes, and is involved in the aetiology of certain human lifestyle diseases, such as Crohn's disease, atherosclerosis and osteoarthritis. The system is able to downregulate stress-related signals that lead to chronic inflammation and certain types of pain, but it is also involved in causing inflammation-associated symptoms, depending on the physiological context. The cannabinoid type-2 (CB_2) receptor, which unlike the CB_1 receptor does not induce central side effects, has been shown to be a promising therapeutic target. While CB_1 receptor antagonists/inverse agonists are of therapeutic value, also CB_2 receptor ligands including agonists are of pharmacological interest. Although the endocannabinoid system is known to be involved in the regulation of energy homoeostasis and metabolism (mainly via CB_1 receptors) there was hitherto no direct link between food intake and cannabinoid receptor activation. Our recent finding that beta-caryophyllene, a ubiquitous lipohilic plant natural product, selectively binds to the CB_2 receptor and acts as a full agonist is unexpected. Maybe even more unexpected is that oral administration of this dietary compound exerts potent anti-inflammatory effects in wild type mice but not in CB_2 receptor ($Cnr2^{-/-}$) knockout mice. Like other CB_2ligands also beta-caryophyllene inhibits the pathways triggered by activation of the toll-like receptor complex CD14/TLR4/MD2, which typically lead to the expression of proinflammatory cytokines (IL-1β, IL-6; IL-8 and TNFalpha) and promotes a TH_1 immune response. In this addendum, the CB_2 receptor-dependent effect of beta-caryophyllene on LPS-triggered activation of the kinases Erk1/2 and JNK1/2 are further discussed with respect to the possibility that both CB_2 inverse agonists and agonists, independent of their G-protein signaling, may block LPS-triggered activation of MAPKs, leading to inhibition of proinflammatory cytokine expression and attenuation of inflammation.

Keywords: cannabinoid CB_2 receptor, MAPK, TLR4, TNFalpha, inflammation, caryophyllene

Apart from the well-known psychomimetic action of the classical cannabinoid Δ9-tetrahydrocannabinol (THC) mediated by centrally expressed cannabinoid CB_1 receptors, it is now generally accepted that the endocannabinoid system also occupies regulatory roles in peripheral tissues.[1–3] The cannabinoid CB_2receptor, which is involved in the tuning of inflammatory processes,[4–6] is able to mediate cellular signals that in many cases lead to attenuation of inflammation.[7,8] The endogenous CB receptor ligands, the arachidonic acid derivatives anandamide and 2-arachidonoylglycerol (2-AG), which are the major endocannabinoids known, partially or fully activate CB receptors in a rather non-selective manner and controlled by strict biosynthesis and degradation.[9]

Anandamide and 2-AG have been shown to inhibit the inflammatory processes triggered upon activation of the toll-like receptor complex CD14/TLR4/MD2 (i.e., LPS and carrageenan stimulation).[10,11] The same effect has also been reported for other CB_2 receptor agonists like JWH133,[12] HU-308,[13] and *N*-alkylamides.[14] Paradoxically, also CB_2 receptor inverse agonists like JTE-907,[15] SR144528,[15,16] and Sch.336,[17] show this effect in the same or similar models. As both CB_2receptor inverse agonists[18] and agonists[7] block identical pathways in an apparently CB_2 receptor-dependent manner, mediators other than cAMP or calcium transients should be involved in this mechanism. In our study,[19] treatment of primary monocytes/macrophages with the CB_2 receptor agonist beta-caryophyllene leads to inhibition of LPS-stimulated phosphorylation of the kinases Erk1/2 and JNK1/2 but not p38, despite the fact that the phosphorylation of these kinases is increased by this compound and JWH133 in resting (non-stimulated) monocytes/macrophages. Thus, the context of differentially activated signaling pathways determines the way signals are integrated. Erk1/2 has been shown to be either activated or inhibited by different GPCRs, both via G-protein dependent and independent mechanisms, involving intracellular calcium transients,[20] the β-arrestin scaffold,[21] and ubiquitination.[22] JNK1/2 is a stress-activated protein kinase that can be modulated by GPCRs. Protein tyrosine kinases are thought to be involved in the regulation of signal transmission from GPCRs to activation of the JNK/SAPK kinase pathway.[23] To inhibit LPS-stimulated Erk1/2 and JNK phosphorylation (i.e., inhibition of their pathways) could be achieved by blocking MyD88, TRAF6 and IKKγ, which would lead to reduced transcription of proinflammatory cytokines and metalloproteinases. The question remains how CB_2 receptor ligands as diverse as inverse agonists and full agonists do the same thing in an apparently receptor-dependent fashion and whether in all cases MAPKs are involved. A direct inhibition of NFκB, which is the primary signal in LPS-induced gene expression, is more unlikely because this factor is inhibited only at higher μM-concentrations of cannabinoids. Thus, how exactly does the CB_2 receptor modulate MAPKs in immune cells? There are different possible mechanisms, such as hitherto neglected CB_2 pathways like the ras-MAPK pathway or even an indirect action via TNFα expression. Interestingly, accumulation of TNFα transcripts, but inhibition of protein expression has been shown after treatment with both CB_2 selective inverse agonists and agonists.[24,25] On the same line, inhibition of the JNK pathway in monocytes/macrophages will inhibit TNFa translation.[26]Moreover, TNFα stimulated pathways could be inhibited indirectly, such that the pro-inflammatory feedback will shut down.

In fact, there is good evidence that constitutive TNFα is inhibited by the endocannabinoid system and that the CB_2 receptor is involved.[27,28] Moreover, TNFα can activate the endocannabinoid system in adipose tissue.[29] As TNFα triggers the activation of Ras, p38 MAPK, ERK1/2, SAPK/JNK and Akt pathways and ultimately proinflammatory cytokine expression and cellular proliferation and migration, it is also possible that the autocrine action of TNFα may be inhibited by cannabinoids. The CB_2 agonists JWH-133 and HU-308 dose-dependently attenuate the effects of TNFα.[30,31] On the other hand, activation of the CB_2 receptor by JWH133 in macrophages has been shown to activate Erk1/2 which leads to expression of IL-10 and thus a counteraction to proinflammatory gene expression.[32] The CB_2receptor has also been found to be a potential target to prevent atherosclerosis,[33] in part via TNFα signalling.[34] Because CB_2 receptor activation attenuates TNFα-induced endothelial cell activation, transendothelial migration of monocytes, and monocyte/neutrophil-endothelial adhesion, and decreases TNFα-induced proliferation and migration of human coronary vascular smooth muscle cells,[35] in addition to inhibiting constitutive and LPS-stimulated TNFα expression, the CB_2 receptor may be an essential pleiotropic modulator of TNFα signal transduction. Providing that the multifunctional cytokine TNFa is involved in numerous pathophysiological processes and the endocannabinoid system can dynamically regulate the levels and effects of this cytokine, more research should be dedicated to the molecular understanding of CB receptor signaling in the immune system.

Unfortunately, the pharmacological tool box for CB_2 receptors is still very limited, the results with selective agonists and antagonists often confusing and the conclusions drawn from such experiments potentially erroneous. For example, it would be wrong to say that 2-AG does mediate intracellular calcium in HL60 cells in a CB_2 receptor-independent manner because the selective CB_2antagonist/inverse agonist AM630 cannot block this effect. The CB_2 inverse agonist SR144528 can exactly do this.[36] In some experiments AM630 and SR144528 are able to block the anti-inflammatory effects of CB_2 agonists; in other experiments SR144528 is anti-inflammatory. Thus, the classical concept of agonists and antagonists, "one lock and one key", does not sufficiently describe cannabinoid GPCR signaling. Even more puzzling is that CB_2 receptor-selective agonists like HU308 increase the inflammation caused by allergens in the skin, while CB_2 antagonists and CB_1 agonists inhibit inflammation,[37] thus pointing to the possibility that different cell types mediate distinct signals. The apparent involvement of CBreceptors and Erk1/2 and JNK MAPKs in the production of inflammatory signals and skin cancer development after UVB irradiation seems to confirm this.[38]

Even though we do obviously not yet understand the underlying molecular mechanisms of these observations, it can be said that in most cases systemic administration of CB_2 receptor-selective ligands leads to attenuation of systemic inflammation in different animal models. It needs to be emphasized, however, that it is currently impossible to determine the molecular mechanism of a ligand in vivo and whether agonists are doing what antagonists should do and vice versa. One hint in that direction comes from data showing that the CB_1 antagonist SR141716A acts like a partial agonist in vivo.[39]

In the case of the dietary natural product beta-caryophyllene, a full CB_2 receptor-selective agonist in vitro, potent anti-inflammatory cannabimimetic effects are observed.[19] Intriguingly, the lowest oral dose tested (5 mg/Kg) of this widespread and apparently non-toxic compound, which is also an FDA-approve food additive, was the most effective. Maybe this strengthens the hypothesis that beta-caryophyllene is indeed a dietary cannabinoid, thus inferring that by eating this compound the endocannabinoid system may be modulated in a beneficial way, possibly through modulation of TNFα pathways, maybe even to such a degree that certain lifestyle diseases could be prevented. The finding that oral beta-caryophyllene is more effective in mice than JWH-133 despite its significantly lower CB_2receptor affinity is encouraging. However, further studies will have to determine whether this natural product is also bioavailable in humans and in more general terms, how CB_2 receptor ligands exactly modulate inflammation in vivo.

Notes

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Footnotes

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