Cannabinoid regulation of microglial activity

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Cannabis sativa preparations (marihuana and hashish) have been used for centuries both, recreationally for their psychotropic effects and as a common remedy. Cannabinoids exert several beneficial actions such as reduction of pain, attenuation of nausea and vomiting, stimulation of appetite, induction of sleep and inhibition of inflammation. Cannabinoids activate their specific receptors, CB1 (expressed mainly in CNS and mediating the psychoactive effects of *Cannabis*), CB2 (expressed mainly in immune cells) and GPR55, a newly described and recently cloned receptor whose function, mechanism of signal transduction and specific ligands are emerging only recently.

Our recent research is focused on the anti-inflammatory properties of cannabinoids. It is well established that prolonged inflammatory processes in the nervous system lead to secondary neuronal damage and contribute to the development of neurodegenerative disorders (e.g. multiply sclerosis, Alzheimer and Parkinson diseases). Growing evidence suggests that cannabinoids diminish neuroinflammatory processes and protect from secondary neuronal damage.

Microglial cells are resident macrophages of the central nervous system and serve as early host defense against pathogens. Paradoxically, chronic activation of microglia is a major factor in the development of neuroinflammation and neurodegeneration. Our group studies the role of cannabinoids in microglial response to inflammatory signals. Using the BV-2 mouse microglial cell line we found that these cells express components of the cannabinoid signaling system including endogenous cannabinoid ligands and cannabinoid receptors (CB2 and GPR55 but not CB1). Moreover, the application of cannabinoids was shown to reduce the secretion of proinflammatory cytokines and interleukins (IL-1β, IL-6, TNF-α) as well as neurotoxic mediators (e.g., NO) from activated microglial cells. These anti-inflammatory properties of the cannabinoids can be correlated with the diminution of neuronal damage. Despite these promising observations, the detailed intracellular mechanisms of the anti-inflammatory activity of the cannabinoids remain unknown.

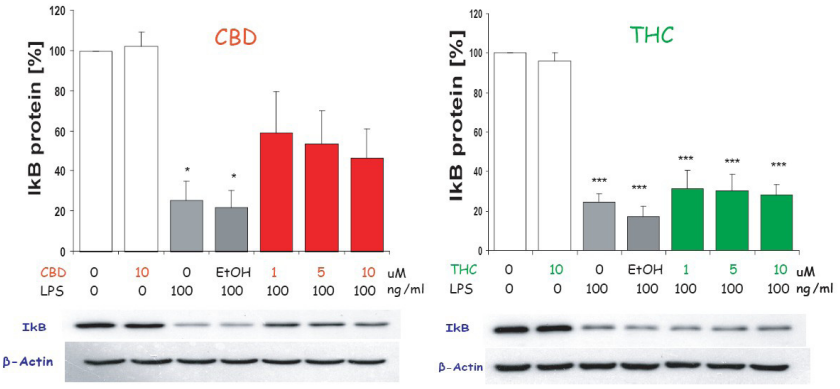

Fig. 1 *CBD, but less so THC, inhibits the degradation of IkB protein (NFkB cytoplasmic inhibitor) in LPS-stimulated BV-2 cells. CBD and THC were dissolved in 10% ethanol and given 2h before LPS. Western blot analysis, β-Actin loading control. * p<0.05, **p<0.005, ***p<0.001 versus control.*

Experimental model of neuroinflammation

In our current studies we have evaluated the signaling pathways and changes in gene expression underlying the anti-inflammatory activity of cannabinoid ligands. The BV-2 mouse microglial cells were activated using *Escherichia coli* lipopolysaccharide (LPS), a bacterial endotoxin that serves as a major constituent of gram-negative bacterial cell walls. The extent of inflammatory reaction was determined based on the production (real time quantitative PCR) and secretion (ELISA) of TNF-α and the interleukins IL-1β, IL-6 and IL-18. Two cannabinoids, both originating from *Cannabis* (phytocannabinoids), have been tested: 9-tetrahydrocannabinol [THC] and cannabidiol [CBD]. These materials differ in their affinity toward the cannabinoid receptors. THC, the main psychoactive constituent of marihuana, binds to both CB1 and CB2 receptors, while CBD, a nonpsychoactive marihuana constituent, lacks affinity for either CB1 or CB2. Both compounds were shown in our previous experiments to decrease the production and release of interleukins in LPS-activated BV-2 microglial cells.

We observed that microglial activation affects the number of CB2 and GPR55 receptors in the cells. However the mode of this regulation depends on the applied inflammatory stimulus: down-regulation after LPS treatment, and up-regulation after interferon-γ, another inducer of microglial activation.

Signaling pathways involved

First, we studied the activity of the cannabinoids on the pathway leading to the activation of the NFkB transcription factor, the primary pathway regulating the expression of inflammatory genes. NFκB is present in the cytoplasm coupled to its inhibitory protein, IκB. IκB stays under the control of upstream interleukin-1 receptor associated kinases, IRAK-1 and IRAK-4 (via the MyD88 adaptor protein dependent pathway). LPS stimulation leads to phosphorylation

Genebank#	Symbol	Gene definition	LPS	CBD+LPS		THC+LPS	
				[fold change vs control]			
Early activation markers							
NM_007782.1	Csf3	colony stimulating factor 3 (granulocyte)	**117.8**	69.1	*(-41 %)*	112.2	*(-5 %)*
XM_132882.1	Cd69	CD69 antigen	**46.5**	14.5	*(-69 %)*	44.3	*(-5 %)*
NM_008039.1	Fpr-rs2	formyl peptide receptor, related sequence 2	**11.7**	3.6	*(-69 %)*	8.9	*(-24 %)*
NM_008198.1	H2-Bf	histocompatibility 2, complement component factor B	**2.4**	1.5	*(-37 %)*	2.4	*(0 %)*
Inflammatory cytokines							
NM_021274	Cxcl10	chemokine (C-X-C motif) ligand 10	**44.0**	23.9	*(-46 %)*	38.9	*(-12 %)*
NM_011331.1	Ccl12	chemokine (C-C motif) ligand 12	**11.0**	2.6	*(-77 %)*	8.3	*(-25 %)*
NM_013654	Ccl7	chemokine (C-C motif) ligand 7	**8.6**	2.5	*(-70 %)*	7.9	*(-9 %)*
NM_013652	Ccl4	chemokine (C-C motif) ligand 4	**8.2**	5.8	*(-29 %)*	7.8	*(-4 %)*
NM_011338	Ccl9	chemokine (C-C motif) ligand 9	**4.9**	1.2	*(-76 %)*	4.7	*(-3 %)*
NM_010510.1	Ifnb1	Interferone beta 1 IFNB1	**11.1**	3.3	*(-70 %)*	6.9	*(-38 %)*
NM_008361	Il1b	interleukin 1 beta	**135.3**	26.4	*(-81 %)*	97.7	*(-28 %)*
NM_010554.1	Il1a	interleukin 1 alpha	**22.9**	7.3	*(-68 %)*	18.1	*(-21 %)*
NM_031168.1	Il6	interleukin 6	**11.2**	7.7	*(-31 %)*	9.3	*(-18 %)*
NM_145636.1	Il27	interleukin 27	**7.7**	2.9	*(-62 %)*	6.8	*(-12 %)*
NM_008360.1	Il18	interleukin 18	**4.1**	2.2	*(-46 %)*	3.8	*(-7 %)*
NM_170704.1	Tnfrsf5	tumor necrosis factor receptor superfamily, member 5	**35.0**	23.6	*(-33 %)*	33.6	*(-4 %)*
NM_009404.1	Tnfsf9	tumor necrosis factor (ligand) superfamily, member 9	**2.6**	1.3	*(-49 %)*	2.7	*(4 %)*
Cytokine responsive/regulated elements							
NM_017466.3	Ccrl2	chemokine (C-C motif) receptor-like 2	**32.7**	22.2	*(-32 %)*	41.4	*(27 %)*
NM_015783.1	G1p2	interferon, alpha-inducible protein	**18.3**	11.6	*(-37 %)*	16.8	*(-8 %)*
NM_010501.1	Ifit3	interferon-induced protein with tetratricopeptide repeats 3	**7.6**	5.3	*(-31 %)*	8.0	*(5 %)*
NM_008326.1	Ifi1	interferon inducible protein 1	**6.5**	4.2	*(-35 %)*	7.3	*(13 %)*
NM_025378.1	Ifitm3	interferon induced transmembrane protein 3	**4.1**	2.3	*(-44 %)*	3.7	*(-10 %)*
NM_016850.1	Irf7	interferon regulatory factor 7	**4.1**	2.3	*(-44 %)*	4.3	*(6 %)*
NM_023141.1	Tor3a	torsin family 3, member A	**2.8**	1.8	*(-36 %)*	2.6	*(-9 %)*
NM_010215.1	Il4i1	interleukin 4 induced 1	**10.0**	3.7	*(-63 %)*	8.1	*(-19 %)*

Table 1 *Microarray analysis showing that CBD, but less so THC, decreases the expression of many LPS-upregulated inflammatory genes in microglial cells. BV2 cells were treated with CBD or THC (10uM) and 2 h later activated with LPS (100ng/ml, 4 h). The results are presented as fold change versus control. The reduction by CBD and THC of the values observed following LPS treatment is expressed in percent values. Neither CBD nor THC given alone affected the expression of these selected genes.*

and subsequent degradation of IRAK1 and of IκB. IκB degradation frees NFκB from the inhibitory complex allowing the translocation of NFkB to the nucleus and the induction of the expression of inflammatory proteins (e.g. interleukins). We observed that 15 min treatment with LPS at 100 ng/ml induced profound IRAK-1 and IκB degradation in BV-2 microglial cells. Both effects of LPS were diminished when the cells were preincubated for 2 h with CBD (1, 5 or 10 μM). Figure 1 shows the results for IkB levels. It is interesting to note that pretreatment with THC (1, 5 or 10 μM) had only minor effects (compared with the large effects of CBD). These results demonstrate that these two cannabinoids differ in their mode of regulation of microglial activity.

Gene expression studies

We applied gene profiling (Illumina microarrays) to follow the changes in gene expression of BV-2 cells exposed to LPS in the presence of THC or CBD. We found that LPS stimulation robustly upregulated the expression of various inflammatory genes such as early activation markers, elements of signaling pathways and a number of inflammatory cytokines and chemokines. Table 1 shows that CBD reversed (by 30 to 80%) many of these LPS-induced changes in gene expression while THC had a relatively minor effect. Interestingly, CBD (but less so THC) given alone, affects the expression of several genes which serve as negative modulators of the inflammatory response. For example, Trib3 gene (tribbles homolog 3, NM_144554.1) whose product is an inhibitory modulator of NFκB pathway, was highly upregulated in CBD-treated and in CBD+LPS treated cells (~15-fold change in both groups). Thus, it seems that CBD may sensitize/prime anti-inflammatory pathways even prior to microglial activation with LPS.

We are currently validating the changes in gene expression using quantitative real time PCR. In addition we plan to follow protein products of the selected genes to allow better understanding of the cannabinoid effects on activated microglia.

In conclusion, although both THC and CBD exert inhibitory effects on the production of inflammatory cytokines in microglial cells, their activity seem to involve different pathways. Our studies should advance the understanding

of the anti-inflammatory activity of cannabinoid ligands and deepen our knowledge about the mechanisms involved in inflammatory processes in the CNS.

Selected publications

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