Cannabinoids differentially modulate leptin signalling in intestinal epithelial cells under inflammatory conditions

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Rationale: Leptin is an important cytokine involved in lipid metabolism and inflammation in the periphery. Increased intestinal leptin in Inflammatory Bowel Disease (IBD) is thought to augment this inflammatory setting through increases in pro-inflammatory cytokine production and up-regulation of nuclear factor kappa B (1). Leptin signalling is principally through the JAK-STAT pathway, although modulation of mammalian Target of Rapamycin (mTOR) has also been documented (2). The activity of mTOR has important downstream targets, such as protein translation, but, more recently, it has been found to impact on autophagy, a process thought to be dysregulated in IBD. Inactivation of mTOR during starvation leads to the induction of autophagy, but less is known about this signalling under inflammatory conditions. Using fully differentiated Caco2 intestinal epithelial cells as an in vitro model, the intersection of leptin and inflammatory cytokine signalling was examined in relation to the mTOR signalling pathway and autophagy. Cannabidiol (CBD), a plant-derived cannabinoid, and N-(2-Chloroethyl)-5Z,8Z,11Z,14Z-eicosatetraenamide (ACEA), a synthetic cannabinoid receptor -1 (CB1R) agonist, were used to explore their ability to limit leptin signalling in this context.

Methods: The following cannabinoids were used in these experiments: ACEA (10^{-7}M), CBD (10^{-6}M). Cytokines used were leptin (100ng/ml) and interleukin (IL)-1beta (10ng/ml). Phosphorylation of mTOR was assessed by immunoblotting using phospho-mTOR antibody (1:1000, Cell Signaling #2971). Autophagy was assessed by immunoblotting Light Chain (LC3B, 1:1000, Cell Signaling #2775), a protein that becomes associated with autophagosomes; and by detection of dansylcadaverine (MDC, 5x10^{-5}M, Sigma #30432), an autofluorescent marker that accumulates in autophagic vacuoles.

Results: Inflammatory cytokine IL-1beta increases autophagy marker, LC3. Both ACEA and leptin inhibit cytokine-induced LC3, whereas CBD enhances autophagy. Under these conditions, ACEA limits leptin-induced phosphorylation of mTOR. Paradoxically, leptin reduces basal autophagy under non-inflamed conditions, an effect limited by ACEA.

Conclusions: A fully functioning autophagy process is required for normal immune responses. Under inflammatory settings, the CB1 receptor agonist, ACEA, limits leptin signalling, but not the downstream function, autophagy. CBD enhances autophagy under these conditions, suggesting differential mechanisms between the two cannabinoids, particularly under inflammatory conditions.