Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol10Issue3abst345P.pdf

C065

CB2 receptor expression is not increased in adipose tissue macrophage during obesity but mediates pro-inflammatory actions.

A Gambero, EMF Gotardo, AN DosSantos, ML Ribeiro. *São Francisco University, Clinical Pharmacology and Gastroenterology Unit, Brazil*

Aims: Endocannabinoid system comprises CB1 and CB2 receptors and, synthesis or metabolism enzymes for anandamide and 2-arachidonoylglycerol endogenous mediators. Obese animals and humans seem to over activate this system in adipose tissue (AT) during obesity. The endocannabinoid system also modulates inflammatory responses through CB2 activation in immune cells. Thus, we investigated if endocannabinoid system expression is altered in AT and in macrophages infiltrated in AT (ATM) from high-fat diet mice. We also analyzed the CB2 cannabinoid agonist actions in macrophage/adipocyte co-culture system and in macrophage migration, *as in vitro* models of AT inflammation.

Methods and Results: Male Swiss mice were feed with high-fat diet (HFD) or standard diet (N) during 12 or 24 weeks (n=12/group). Body weight and glucose homeostasis (glucose basal and insulin test tolerance) were evaluated. Whole epididymal AT and isolated macrophages ($CD11b^+$ cells) were collected for gene expression analysis by qRT-PCR (*CB1 receptor*, *CB2 receptor*, *N-acyl-phosphatidylethanolamine (NAPE), fatty acid amide hydrolase (FAAH), diacylglycerol lipase (DAGL*)). 3T3-L1 adipocytes and RAW 264.7 macrophages were co-cultured in presence or absence of CB2 agonist JWH-015 (1, 3 and 10 µM). TNF-α and IL-10 was measured by ELISA in co-cultures stimulated or not by lipopolysaccharide (LPS, 1 ng/ml). RAW macrophages were also employed in migration assays using modified Boyden Chamber (25 ng/ml MCP-1 as stimulus). Comparisons among groups were performed using ANOVA followed by Student's t or Dunnett's multiple comparisons test. HFD mice presents high body weight and insulin resistance, confirming the obesity status. CB2 receptor gene expression in AT was higher in obese mice after 12 or 24 weeks (14.0±3.3 and 3.3±0.4 arbitrary unit (AU) for HFD and C 12 weeks group, respectively ($p<0.05$); 19.8±2.1 and 11.3±2.0 AU for HFD and C 24 weeks group, respectively ($p<0.05$)). NAPE expression was reduced only after 24 weeks in AT (15.5±4.2 and 38.8±5.8 AU for HFD and C 24 weeks, respectively ($p<0.05$)). When isolated ATM from lean and obese mice were analyzed no differences were observed in CB2 receptor or NAPE expression. LPS-induced TNF-α release in the co-culture system was increased by JWH 015 (17.80±0.53 and 32.65±0.10 pg/mg protein for LPS and JWH015 3µM, respectively ($p<0.05$)). Basal and LPS-stimulated IL-10 was decreased in supernatant of co-cultures (0.42±0.08 and 0.16±0.01 pg/ml of IL-10 for basal and JWH015 10µM; 2.42±0.14 and 0.40±0.06 pg/mg protein of IL-10 for LPS and JWH015 10µM, respectively ($p<0.05$)). JWH 015 increase spontaneous cell migration and seems to act as chemoattractant substance at 1 and 3 µM, respectively ($p<0.05$). No changes in MCP-1-induced RAW macrophage migration as observed when cells were treated with JWH 015.

Conclusion: Increased CB2 expression in AT from obese mice could be due an increased macrophage infiltration, because in isolated cells this alteration was not detected. Anyway, the stimulation of CB2 receptor reduces IL-10 and increases TNF-α release by adipocytes/macrophages. Additionally, CB2 stimulation induces macrophage migration, suggesting a pro-inflammatory action that could be deleterious in the AT inflammation during obesity.