Increased expression of cannabinoid receptor 2 but not μ opioid receptor and its ligand β-endorphin (β-END) correlates with symptoms severity of patients with irritable bowel syndrome (IBS)

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Background and Aims: Irritable Bowel Syndrome (IBS) is a functional bowel disorder characterized by abdominal pain and discomfort and changes in bowel habit. A current experimental hypothesis underlying IBS pathophysiology indicate that an enhanced mucosal permeability is responsible for an increased income of microbial and dietary antigens from the intestinal *lumen* to the intraepithelial compartment, leading to an enhanced immune activation. This, could lead to an altered neuro-immune interaction and increased visceral pain perception. The opioid and cannabinoid systems regulate many factors that are likely to be involved in IBS pathophysiology, including motility, secretion, visceral hypersensitivity and immune response. μ opioid receptor (MOR) and its ligand, β-endorphin (β-END) and the cannabinoid receptor 2 (CB2) are expressed in immune cells and the enteric nervous system. The aim of this study was to investigate the expression of μOR, β-END and CB2 in IBS patients with either constipation (IBS-C) or diarrhea (IBS-D) compared to healthy controls (HC) and to evaluate a possible correlation between the expression levels of the biological targets analyzed with patients abdominal pain and symptom severity. **Methods**: Mucosal biopsies were obtained from the sigmoid colon of 23 patients with IBS-C (11 M and 12 F; age 34.4 ± 1.5), 32 patients with IBS-D (16 M and 16 F; age 37 ± 1.9) and 31 HC (12 M and 19 F; age 36.3 ± 2.3) and processed for quantitative RT-PCR, Western blotting and immunohistochemistry. Mouse CD4 and EMR1 antibodies were used as markers for lymphocyte and macrophage, respectively with rabbit μOR or β-END antisera. Patients symptoms severity and abdominal pain were scored following the Bowel Symptom Questionnaire (BSQ). Kruskal-Wallis analysis of variance , Dunn's comparison *post-hoc* test and Pearson's multivariate correlation analysis were applied to statistical data analysis. **Results:** μOR mRNA and protein expression was increased in IBS patients compared to HC without significant differences among the subgroups ($p=0.07$). β-END immunoreactivity (IR) was increased in IBS overall ($p=0.01$) and in IBS-C ($p<0.05$) compared to HC. CB2 mRNA expression was markedly higher in IBS overall ($p<0,05$) and in IBS-C (~7 fold increase vs. HC $p<0.05$). β-END-IR was localized to CD4+ lymphocytes as well as on EMR1 positive macrophages. μOR-IR was localized to CD4+ lymphocytes and epithelial cells. Finally, correlation analysis indicate that the observed increase of CB2 receptor protein correlates with patients symptom severity ($cor=0,49$; $p<0.05$) but not with abdominal pain severity, no other correlation were detected between patients symptoms and the quantitative biological parameters observed. **Conclusions**: These data show an increase of the expression of both β-END and μOR, with a more pronounced upregulation of β-END in IBS-C, and that both receptor and ligand are prominently localized to immune cells. CB2 was markedly increased in patients with IBS, particularly in IBS-C. The increase of CB2 protein content correlates with patients symptom severity scores. The present findings support a contribution of opioid and cannabinoid endogenous systems to IBS pathophysiology and may pave the way for a novel therapeutic strategy for IBS.