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The anti-inflammatory effects of palmitoylethanolamide and cannabidiol in the human colon are mediated by PPARα , CB_2 and TRPV1

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INTRODUCTION: We have previously shown that palmitoylethanolamide (PEA) and cannabidiol (CBD) have an anti-inflammatory effect in experimentally inflamed normal colonic tissue, in explants from active inflammatory bowel disease and explants from acute appendicitis (Alhamourni et al. 2010). In this study we sought to identify through which receptors these drugs act in an experimentally inflamed human colonic explant model of colitis.

METHODS: Ethical approval was gained via the local Health Research Authority. Samples of normal human appendix (n=9) were obtained from elective bowel cancer resections after giving informed consent. Sections of mucosa (2x2mm) were incubated in culture media (Eagles minimum Essential Media with 10% foetal bovine serum and 1% non-essential amino acids, Sigma Aldridge). After 24 hours media was changed and samples were treated with inflammatory cytokines (IFNγ (10ng/ml) for 18 hours, followed by TNFα (10 ng/ml) for 6 hours) in the presence of PEA (10μM), CBD (10μM) or vehicle (0.01% ETOH). The effect of PEA or CBD on IL-8, IL-6 and monocyte chemoattractant protein 1 (MCP)-1 secretion (with or without inflammatory cytokines) was measured via ELISA (R&D systems). Sites of action of PEA and CBD we investigated by co-applying the following antagonists with PEA or CBD under inflammatory conditions: AM251 100nM (CB_1 antagonist), AM630 100nM (CB_2 antagonist), GW6471 500nm (PPARα antagonist), GW9662 100nM (PPARγ antagonist), SB366791 500nM ($TRPV_1$ antagonist), and CID16020046 500nM (GPR55 antagonist) (all Tocris Bioscience, Bristol, UK). Cytokine concentrations were corrected for total protein content with bicinchoninic acid assay. Data was analysed with repeated measures ANOVA.

RESULTS: Stimulation of colonic tissue caused a significant increase in the production of IL-8, MCP-1 ($p<0.05$) and IL-6 ($p<0.01$). These increases were prevented by treatment with PEA and CBD across all measured cytokines ($p<0.01$). The anti-inflammatory effect of PEA on cytokine production was prevented by the PPARα antagonist GW6471 ($p<0.05$). The effect of CBD was prevented by the CB_2 antagonist AM630 ($p<0.05$) and the TRPV1 antagonist SB366791 ($p<0.05$).

CONCLUSIONS: PEA and CBD inhibit the inflammatory response in ex vivo human colonic tissue. These effects are mediated by PPARα and CB_2 with TRPV1 respectively.

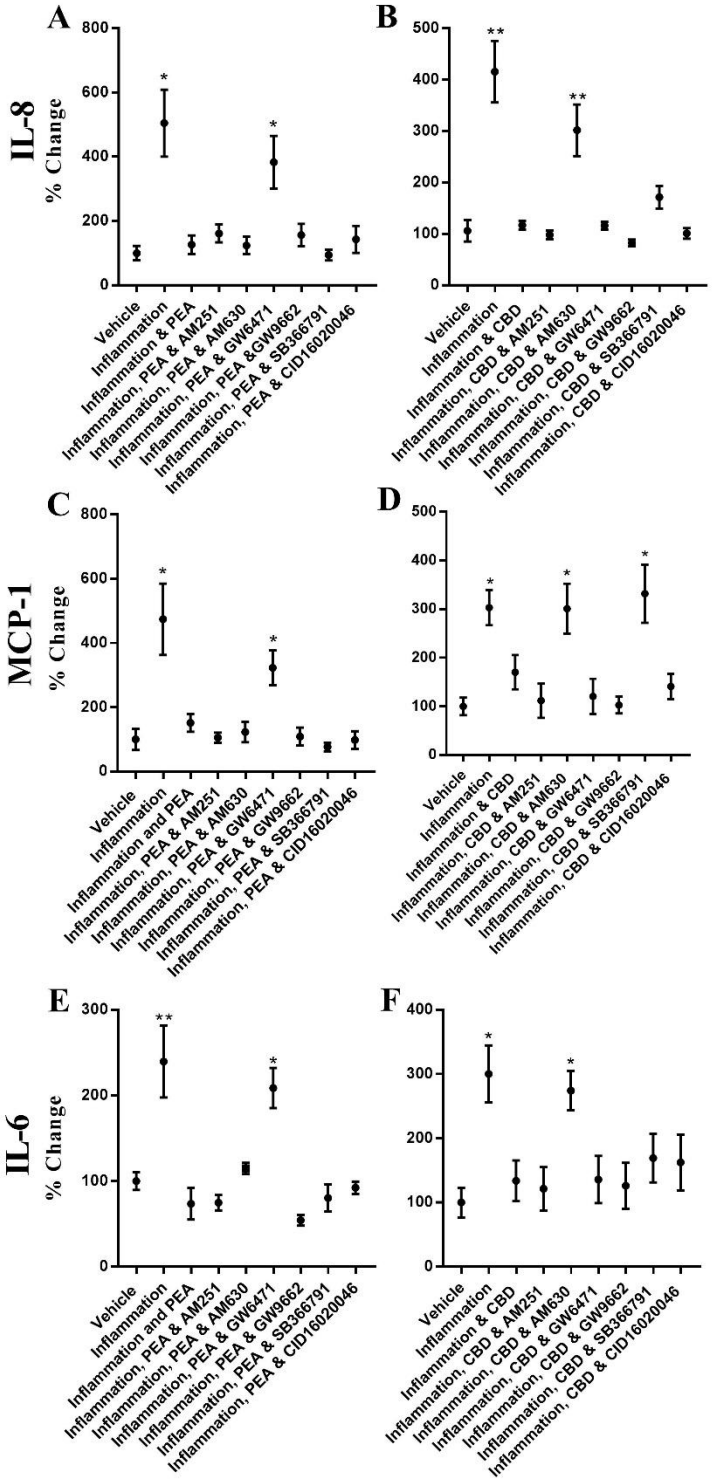

Figure 6: The effects of PEA (A,C,E) and CBD (B,D,F) on the secretion of IL-8, MCP-1 and IL-6 in response to an inflammatory protocol in explant human colonic tissue in the presence of receptor antagonists, measured by ELISA (compared by repeated measures ANOVA, n=7). Data presented as mean +/- SEM per condition. Asterixes (*) represent significant difference from vehicle (*<0.05, **<0.01).