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The Pharmacological Effects Of Palmitoylethanolamide (PEA) On Intestinal Permeability In Vitro Using Caco-2 Cell

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INTRODUCTION: Gut integrity and normal intestinal permeability (IP) is essential for health. Numerous conditions such as shock and inflammatory bowel disease are associated with increased gut permeability. Endocannabinoids are present in many organs including the GI tract. We had previously reported that endocannabinoids modulate IP. PEA is an important endocannabinoid in food regulation and inflammation. We hypothesised that PEA might affects IP and play a role in modulating IP in inflammation. The aim of this study was to examine whether PEA modulates IP *in vitro*.

METHODS: Caco-2 cells (a standard in vitro model of gut mucosa) were grown on cell culture inserts to confluence. Trans-epithelial electrical resistance (TEER) was measured as a standardized measurement of permeability. Inflammation was modelled by addition to 3 ng/ml^{-1} or 10 ng/ml^{-1} of interferon-gamma (INFγ) and tumour necrosis factor-alpha (TNFα) for 24h. PEA was applied either to apical or basolateral aspects of cells. The potential target sites of PEA were probed using receptor antagonists; AM251 (CB_1), AM630 (CB_2), GW9662 (Peroxisome proliferator-activated receptor- gamma or PPARγ), GW6471 (Peroxisome proliferator-activated receptor- alpha or PPARα) (all 100 nM), capsazepine (transient receptor potential vanilloid subtype-1 or TRPV1) and O-1918 (proposed epithelial cannabinoid receptor) (1μM). The role of fatty acid amide hydrolase (FAAH) on the effects of PEA on IP was investigated. PEA was applied to apical or basolateral compartments in the absence or presence of FAAH inhibitor (URB597). In some studies, this was combined with antagonists. Appropriate vehicles (ethanol and dimethylsulphoxide or DMSO 0.01%) were applied to the control inserts. Resistance readings were taken over 48h. Data was analysed using ANOVA with Dunnett's post-hoc test. A significance level of $P< 0.05$ was considered as statistically significant.

RESULTS: In normal conditions, apical application of PEA transiently decreased permeability via PPARα. Basolaterally, PEA also decreased permeability via PPARα (n=3; $P<0.001$). Inhibition of PEA degradation, using fatty acid amide hydrolase (FAAH) inhibitor (URB597) caused further increases in resistance. In the inflammation model, basolateral application of PEA reversed the increased permeability caused by cytokines. When PEA was added to the basolateral compartment at the same time as the cytokines, it inhibited the fall in TEER (increased permeability) (n=3; $P<0.01$). These PEA effects were inhibited by GW6471. PEA had no significant effect in inflammation when applied to the apical membrane.

CONCLUSION: This study demonstrates that in normal and inflammatory conditions, PEA administration to the basolateral compartment causes decreased permeability *in vitro*, mediated by PPARα. Inhibiting PEA degradation causes further decreases permeability. PEA modulates IP in normal and inflammatory states and suggests potential therapeutic uses for disorders linked with increased IP such as shock or inflammatory bowel diseases.