Oleylethanolamine and palmitoylethanolamine modulate intestinal permeability in vitro via TRPV1 and PPARα

Mustafa A. Karwad,* Tara Macpherson,‡ Bo Wang,* Elena Theophilidou,* Sarir Sarmad,# David A. Barrett,# Michael Larvin,*§ Karen L. Wright,† Jonathan N. Lund,* and Saoirse E. O’Sullivan*†

*School of Medicine, Royal Derby Hospital, and ‡Centre for Analytical Bioscience, School of Pharmacy, University of Nottingham, Nottingham, United Kingdom; †Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Lancaster, United Kingdom; and #Graduate Entry Medical School and †Health Research Institute, University of Limerick, Limerick, Ireland

The human gastrointestinal (GI) tract forms the largest interface between the external environment and internal milieu (1). Aside from its digestive functions, it constitutes the most complex and evolved element of immune defense. Intestinal epithelial cells, together with their mucous coatings, constitute a protective barrier, across which paracellular permeation is selectively regulated by transmembrane protein contractility within the intercellular tight junctions (2, 3), thus preventing the loss of water and solutes from the gut, while simultaneously permitting the absorption of water and nutrients, but preventing the ingress of inflammatory mediators. OEA and PEA have endogenous roles and potential therapeutic applications in conditions of intestinal hyperpermeability and inflammation.—Karwad, M. A., Macpherson, T., Wang, B., Theophilidou, E., Sarmad, S., Barrett, D. A., Larvin, M., Wright, K. L., Lund, J. N., O’Sullivan, S. E. Oleylethanolamine and palmitoylethanolamine modulate intestinal permeability in vitro via TRPV1 and PPARα. J. FASEB 31, 469–481 (2017).

www.fasebj.org

KEY WORDS: gut · cannabinoid · nuclear receptor

ABBREVIATIONS: AEA, arachidonyl ethanolamide or anandamide; AM251, N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; AM630, 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl]-[4-methoxyphenyl] methanone; AQP, aquaporin; Caco, carcinoma colon cell line; CB₁/₂, cannabinoid receptor 1/2; CREB, cAMP response element–binding protein; ECS, endocannabinoid system; EMEM, Eagle’s minimum essential medium; EVOM, epithelial tissue vol-ohm meter; FAAH, fatty acid amide hydrolase; FAK, focal adhesion kinase; FBS, fetal bovine serum; GI, gastrointestinal; GW6471, [25R]-2-[(1Z)-1-methyl-3-oxo-3-[4-(trifluoromethyl]phenyl)]-1-propanyl]laminoo][4-[2-(5-methyl-2-phenyl-4-azolyl]methoxy]phenylpropyl]carboxy acid ethyl ester; GW9662, 2-chloro-5-nitro-N-phenylbenzamide; IBD, inflammatory bowel disease; I–O-1918, 1,3-dimethoxy-5-methyl-2-[(1R)-3-methyl-6-(1-methylhexyl)2-cyclohexen-1-yl]benzene; LC-MS/MS, liquid chromatography–tandem mass spectrometry; OEA, oleylethanolamine; PEA, palmitoylethanolamine; PPAR, peroxisome proliferator-activated receptor; TBS, Tris-buffered saline; TBST, TBS Tween; TRPV, transient receptor potential vanilloid; TRPV1, transient receptor potential vanilloid-1; URB970, 3’-(aminocarbonyl)[1,1’-biphenyl]-3-y]-cyclohexylcarbamate

1 Correspondence: School of Medicine, Royal Derby Hospital, University of Nottingham, Derby, DE22 3DT United Kingdom. E-mail: saoirse.osullivan@nottingham.ac.uk
doi: 10.1096/fj.201500132
This article includes supplemental data. Please visit http://www.fasebj.org to obtain this information.
Impaired intestinal barrier function leading to hyperpermeability is associated with a wide variety of human diseases and conditions, for example acutely in shock and multiple organ–system dysfunction with splanchic ischemia (3), sepsis (2), or, more gradually, including inflammatory bowel disease (7–10), celiac disease (11), irritable bowel syndrome (12, 13), and a range of other conditions (14, 15). Family studies have demonstrated that increased intestinal permeability can precede the clinical presentation of inflammatory bowel disease (16–18). The regulation of intestinal permeability is poorly understood, and improved understanding is necessary for the development of therapeutic interventions specifically targeted at restoring normal permeability (19).

Cannabis sativa plant extracts have been used anecdotally for over 5 millennia for the treatment of GI disorders including nausea, vomiting, anorexia, intestinal inflammation, and diarrhea (20). Endocannabinoids are intercellular lipid signaling molecules derived from arachidonic acid and synthesized on demand from cell membrane precursors. Examples were found to be expressed in the gut only 20 yr ago (21), and subsequently endocannabinoids and their receptors were shown to be key regulators of a variety of GI functions, including emesis (22), intestinal motility (23), and secretion (24). Endocannabinoids play significant roles in inflammation and apoptosis (25, 26) and specifically in intestinal inflammation (27), opening up the possibility of new therapeutic options (28).

Endocannabinoids exert their effects by activation of cannabinoid receptor 1 and 2 (CB1 and CB2) (29) and other target sites of action, such as transient receptor potential ion channels (TRPs) (30), peroxisome proliferator-activated receptors (PPARs) (31), and the orphan GPCRs GPR119 (32) and GPR55 (33). All of these target sites are expressed in the GI tract. These receptors, together with endocannabinoid ligands and the enzymes responsible for their metabolism, are collectively referred to as the endocannabinoid system (ECS). The ECS is involved in modulating GI motility and intestinal inflammation and is up-regulated in intestinal inflammation. Our group has reported that cannabinoids modulate intestinal permeability in vitro in Caco-2 intestinal cells (34, 35), which has also been shown in vivo (36). The plant-derived cannabinoids Δ⁹-tetrahydrocannabinol and cannabidiol reverse the increased permeability caused by EDTA or cytokines via CB₁ activation (34, 35). By contrast, the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) increase permeability of the Caco-2 monolayer via CB₂ (34, 35), and inhibiting their synthesis improves the effects of inflammation on permeability, suggesting that the endogenous production of these compounds in response to inflammation plays a role in promoting permeability changes at the epithelium.

Oleoylthanolamine (OEA) is an endocannabinoid-like compound that does not bind to cannabinoid receptors (37). It is produced on demand in enterocytes, and its production is stimulated by food intake (38) or reduced by food deprivation (39). It is a PPARα agonist (40) that activates TRPV1 channels (41), and the orphan GPCRs GPR55 and GPR119 (42). Administration of OEA suppresses food intake, inhibits body weight gain (43), and induces satiety via PPARα activation (44). It also has a role in regulating lipid metabolism (45) and reduces cholesterol levels in mice via PPARα (46, 47). Palmitoylethanolamine (PEA) is another endocannabinoid-like compound found at high levels in the upper GI tract compared with other organs and tissues (39). It reduces intestinal injury and inflammation in mice via PPARα (47, 48). More recently, oral or intraperitoneal administration of PEA has been found to reduce inflammation and damage in dinitrobenzene sulfonic acid-induced colitis in mice, mediated by PPARα, CB2, and GPR55 (49), and inhibition of the enzyme responsible for PEA degradation also reduces inflammation in 2 mouse models of colitis (50).

In the present study, we hypothesized that OEA and PEA, which often have opposing physiologic actions and pharmacology that differ from that of AEA and 2-AG, may also modulate intestinal permeability and play a role in intestinal inflammation. We hypothesized that these compounds would have a beneficial effect on intestinal permeability based on their positive effects in vivo in simulated inflammation.

MATERIALS AND METHODS

Cell culture

Caco-2 cells (passages 62–86; European Collection of Cell Culture, Fornon Down, United Kingdom) were cultured in a T75 cell culture flask in Eagle’s minimum essential medium (EMEM), supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and l-glutamine at 37°C in 5% CO₂ and 95% air.

Effects of OEA and PEA on Caco-2 monolayer permeability

The cells were seeded at 20,000 cells on 6.4 mm diameter, 0.4 µm pore size polyethylene terephthalate inserts (BD Falcon Biosciences, Oxford, United Kingdom) and grown for 14–18 d. Trans-epithelial electrical resistance (TEER) was measured with an epithelial volt-ohm meter (EVOM²; World Precision Instruments, Sarasota, Fl, USA) as an indicator of cellular permeability. Caco-2 cell monolayers with TEER value >1000 Ω · cm² were used. Caco-2 cell monolayers were washed twice in HBSS (+N-2-hydroxyethylpintential permeabilityrnazine-N-2-ethanesulfonic acid, or HEPES and penicillin/streptomycin), and the baseline TEER was measured. Increasing concentrations of OEA or PEA (1 nM–10 µM) or vehicle (0.1% ethanol) were applied in prewarmed EMEM to the apical or basolateral compartment of inserts, and TEER was measured over the next 48 h.

The following target sites of action were investigated (receptor antagonist and concentrations shown in brackets): CB₁ [AM251, 100 nM (Ki 7.49 nM)], CB₂ [AM630, 100 nM (Ki 31.2 nM)], PPARγ [GW9662, 100 nM (IC₅₀ 3.3 nM)], PPARα [GW6471, 100 nM (IC₅₀ 240 nM)], TRPV1 [capsazepine, 1 µM (Ki 3.2 µM)] and the proposed endothelial cannabinoid receptor antagonist (O-1918, 1 µM). In some experiments, OEA and PEA (3 µM) were applied with an inhibitor of their degradation by fatty acid amide hydrolase (FAAH), using URB597 (1 µM), in the absence and presence of capsazepine or GW6471.
To simulate inflammatory conditions, 10 ng/ml IFNγ was added basolaterally. After 8 h, 10 ng/ml TNFα was added for another 16 h. OEA and PEA were added to the apical or basolateral compartment at various time points, either at the same time as IFNγ (time 0 h, to potentially block the development of inflammation) or after the induction of inflammation or at 24, 48, or 72 h, to potentially limit the inflammatory increase in permeability. In some experiments, this protocol was performed in the presence of antagonists. For prolonged (chronic) inflammatory studies, repeated applications of 3 ng/ml of IFNγ and TNFα were used.

Cell viability assays

To test the effects of OEA and PEA on cell viability in fully differentiated Caco-2 cells, the cells were brought to confluence and maintained in complete medium for up to 18 d in 96-well plates. A concentration response to OEA and PEA was then performed in complete medium over 48 h, after which PrestoBlue Reagent (Thermo Fisher Scientific, Waltham, MA, USA) was added directly to the cell culture (1:10). After 10 min, absorbance was measured with excitation at 570 nm with 600 nm as the reference wavelength for normalization. Data were calculated as the mean percentage change from untreated control.

To test the effects of OEA and PEA on cell viability in proliferating Caco-2 cells, we seeded 5 × 10⁵ cells in quadruplicate into a 96-well microplate in standard medium with 8% serum. A concentration response to OEA and PEA was then performed in complete medium over 48 h, after which PrestoBlue Reagent (Thermo Fisher Scientific, Waltham, MA, USA) was added directly to the cell culture (1:10). After 10 min, absorbance was measured with excitation at 570 nm with 600 nm as the reference wavelength for normalization. Data were calculated as the mean percentage change from untreated control.

Phalloidin staining

Phalloidin is an F-actin stain that allows for the visualization of the structure and inferred function of this cytoskeletal filament protein. Cells are fixed first so the image is a snapshot of the given timepoint. Linear actin fibers (mostly parallel) are the polymerized F-actin in the cytoplasm and can be quite pronounced at the timepoint. Linear actin fibers (mostly parallel) are the polymerized F-actin in the cytoplasm and can be quite pronounced at the timepoint.

Potassium channel activation

To test the ability of OEA and PEA to modulate potassium channels in Caco-2 cells, the FluxOR Potassium ion channel assay (Thermo Fisher Scientific) was used. In brief, Caco-2 cells were grown on 96-well plates until fully confluent and differentiated. The cells were loaded with the nonfluorescent, thallium-specific dye and treated apically with increasing concentrations of OEA or PEA. When potassium channels are stimulated, thallium-specific FluxOR dye and treated apically with increasing concentrations.
centrifugation, and evaporation. Before analysis, each sample extract was reconstituted in acetonitrile. An MDS SCIEX 4000 Q-Trap hybrid triple-quadrupole-linear ion trap mass spectrometer (Thermo Fisher Scientific), operated in positive electrospray ionization mode, was used in conjunction with a series 10AD VP LC system (Shimadzu, Columbia, MD, USA), with an ACE 3 C8, 100 × 2.1 mm, 3 μm particle size column (Advanced Chromatography Technologies Ltd., Aberdeen, United Kingdom). Quantification was performed by measuring specific OEA and PEA precursor and product ions together with a calibrated internal standard method.

Chemicals and reagents

All chemicals and reagents used in these experiments were purchased from Sigma-Aldrich, unless otherwise stated. OEA and PEA and the receptor antagonists AM251, AM630, GW9662, GW6471, capsaizepine, and O-1918 were purchased from Tocris (Bristol, United Kingdom). OEA and PEA were dissolved in ethanol to 10 mM, with further dilutions made in EMEM. All receptors antagonists were dissolved in DMSO to 10 mM, with further dilutions in EMEM. IFNγ (100 μg) and TNFα (50 μg) were purchased from Thermo Fisher Scientific, and dilutions were made in FBS.

Statistical analysis

Results are expressed as means ± SEM. Time-course data were compared by 2-way, repeated-measures (repeated by time factor) ANOVA, with Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA). Statistical significance between manipulations and vehicle controls were determined by Dunnett’s post hoc test. Results reaching P < 0.05 were statistically significant.
RESULTS

Permeability studies

Our initial experiments sought to explore whether the N-acylethanolamines were able to modulate the ionic conductance of the paracellular pathway, as a proxy for tight junction integrity. When applied to the apical membrane compartment, OEA increased Caco-2 cell monolayer transepithelial electrical resistance (TEER) (i.e., decreased permeability) in a concentration-dependent manner significantly different from control at 1, 3, and 10 \( \mu M \) (Fig. 1A). When applied to the basolateral membrane, OEA decreased TEER (i.e., increased permeability) in a concentration-dependent manner at 1, 3, and 10 \( \mu M \) (Fig. 1C). The log EC\textsubscript{50} of OEA at the apical membrane was \(-5.43\) and at the basolateral membrane was \(-5.92\) (Supplemental Fig. 1A).

PEA caused a large increase in TEER when applied to the apical membrane at 1, 3, and 10 \( \mu M \) (Fig. 1B). Although transient, the effects of 10 \( \mu M \) PEA remained significantly above the effect of vehicle until 48 h after administration. When applied to the basolateral membrane, PEA increased resistance from 30 min after application in a concentration-dependent manner from 1 \( \mu M \) (Fig. 1D). From 8 h on, after application, a significant effect of 300 nM PEA was observed. The log EC\textsubscript{50} of OEA at the apical membrane was \(-5.43\); at the basolateral membrane, it was \(-5.92\) (Supplemental Fig. 1B, C).

To ensure that these changes in permeability were not related to changes in the number of cells, we performed cell viability assays. Neither OEA nor PEA affected Caco-2 cell viability in fully confluent (Supplemental Fig. 2A) or proliferating (Supplemental Fig. 2B) cells. In addition, the expression of 2 AQPs found in mammalian intestines (AQP3 and -4) that transport water, glycerol, ammonia, and hydrogen peroxide (52) and could affect membrane permeability, was investigated. Apical treatment of Caco-2 cells with either OEA or PEA (10 \( \mu M \), 1 h) led to a significant reduction in the membrane expression of AQP3 (Fig. 1E) and AQP4 (Fig. 1F). Furthermore, changes in transmembrane ion gradients generate osmotic alterations that can affect cell volume, and the involvement of potassium ion influx in cell volume regulation has only recently been recognized (53). Apical treatment of Caco-2 cells with either OEA or PEA led to a concentration-independent increase in fluorescence indicative of activation of potassium channels (Fig. 1G).

Cytoskeletal changes

To clarify the impact of these lipid mediators on cytoskeletal changes, we treated and processed mature Caco-2 cells, to visualize F-actin (Fig. 2). At the apical focal plane, cell-to-cell adhesion is visible across all treatments with no gaps. OEA rendered the cortical F-actin to have an irregular morphology (top, middle) compared to vehicle control (top left), whereas PEA induced focal adhesion plaques at sites of cell-to-cell adhesion (top right). Cell adhesion to the slide can be seen at the basal focal plane in resting cells (bottom left), with OEA inducing a loss of both cellular tension through reduced actin filaments and focal adhesions (bottom middle). On the other hand, PEA caused an increase in polymerized F-actin filaments and focal adhesion plaques (bottom right). These occur throughout the cytoplasm of the cell, as well as some cortical accumulation. Images in Fig. 3 are representative fields of view from 4 separate experiments. Full \( z \) stacks projected into single images can be viewed in Supplemental Fig. 3.

Intracellular signaling

Because the action of the contractile cytoskeleton enables the cellular changes required to adjust permeability in

![Figure 2. F-actin cytoskeletal networks in mature Caco-2 cells. Cells were grown to confluence and fully differentiated on glass chamber slides. Fresh complete medium was applied before the addition of compounds for 1 h. Cells were then fixed and stained with phalloidin and DAPI, and images were captured by confocal microscopy. Left: vehicle control (VC, ethanol); middle: OEA (10 mM); right: PEA (10 mM). From a total of 26 \( z \) stacks, the top panels are representative images of an apical focal plane and bottom panels of basal focal planes taken as close to the adhesion surface (glass slide) as possible. Images are representative fields of view from 4 separate experiments. Full \( z \) stacks projected into single images can be viewed in Supplemental Fig. 2. Scale bar, 10 mm.](image-url)
response to its environment, we investigated the signaling events known to be important for cytoskeletal modifications—namely, FAK and the p42/44 MAPKs (54). OEA induced a transient increase in both FAK and Erk1/2 (Fig. 2, left panel, top and third blot down), peaking at 5 min and returning to basal levels by 30 min (Fig. 3B, C). PEA induced phosphorylation of Erk1/2 to significantly higher levels than OEA (Fig. 3A, right panel, third blot down), but FAK activation by PEA continued to increase up to 1 h after application (Fig. 3B, C, right panel, top blot).

We performed further experiments using Luminex technology and commercially available panels for multiple pathways and the SRC pathway. As seen with Western blot analysis (Fig. 1A), OEA significantly increased phosphorylated ERK1/2 and also p70s6K, CREB and NF-κB, and significantly decreased phosphorylated p38 and JNK (Fig. 3D). PEA significantly increased phosphorylated ERK1/2, p70s6K, and CREB, and significantly decreased phosphorylated p38 (Fig. 3E). In this panel, significant differences between OEA and PEA were observed in the ERK1/2 and Akt response (Supplemental Fig. 4D, F). In the Src family panel of signaling proteins, OEA and PEA significantly reduced phosphorylated Src, Yes, Lck, Lyn, Fgr, and Blk (significance is not shown in Fig. 3F and G; for clarity, please refer to Supplemental Fig. 5). OEA also significantly reduced phosphorylated Fyn and Hck. The reduction was more pronounced at 10 min for OEA (Fig. 3F) and at 2 min for PEA (Fig. 3G).

**Receptor mechanism of action**

The ability of a submaximal concentration of OEA (3 μM, apical application) to increase TEER was inhibited by capsazepine (a TRPV1 antagonist) only (Fig. 4A). The ability of OEA (3 μM, basolateral) to decrease TEER was inhibited by the TRPV1 antagonist capsazepine and the PPARα receptor antagonist GW6471 (Fig. 4C). The effect of PEA at the apical membrane was inhibited by the PPARα antagonist GW6471 (Fig. 4B). The effect of PEA at the basolateral membrane was inhibited by a PPARα antagonist (Fig. 4D).

OEA and PEA are degraded by FAAH. When OEA or PEA were applied in combination with the FAAH inhibitor URB597, their effects were amplified. OEA (3 μM, apically) caused further increases in TEER when coapplied with an FAAH inhibitor (URB597; Fig. 5A) to the apical membrane, and this effect was inhibited by TRPV1 antagonism. OEA (3 μM) also caused a further decrease in resistance when coapplied with URB597 to the basolateral membrane, via TRPV1 and PPARα (Fig. 5C). PEA (3 μM) caused further increases in resistance when coapplied with URB597 (at either the apical or basolateral membrane), and this resistance was inhibited by the PPARα antagonist GW6471 (Fig. 5D).

**Effects of OEA and PEA on cytokine-induced hyperpermeability**

When applied to the apical membrane concurrently with cytokines, OEA (3 μM) prevented the fall in TEER (Fig. 6A). Apically, OEA also recovered the increased permeability when applied 24 h after cytokines (Fig. 5B). By contrast, application of OEA to the basolateral membrane (at either time 0 or 24 h) caused further decreases in TEER than was caused by cytokines alone, indicating further increased permeability (Fig. 6A, B).
PEA (3 μM) prevented the decline in TEER caused by cytokines when applied at the same time to the basolateral membrane, evident as early as 8 h into the cytokine exposure (IFNγ exposure only, Fig. 6A). This effect of PEA at the basolateral membrane was still observed when PEA was applied 24 h after exposure to cytokines (Fig. 6B).

However, PEA has no effect on cytokine-increased permeability when applied to the apical membrane at either time point (Fig. 6A, B).

To establish whether OEA and PEA are produced endogenously in cells in response to simulated inflammatory conditions, we measured the cellular and secreted levels of these compounds by LC-MS/MS, using the inflammation protocol used to assess permeability changes. Cellular levels of OEA (P < 0.001; Fig. 6C) and PEA (P < 0.01, Fig. 5E) were significantly increased by the inflammatory protocol. Significantly raised levels of OEA (P < 0.0001, Fig. 6D) and PEA (P < 0.0001, Fig. 6F) were also detectable in the medium in response to simulated inflammation.

### Mechanisms of action of OEA and PEA on cytokine-induced hyperpermeability

When applied to the apical membrane concurrently with cytokines, as before (Fig. 6), OEA (3 μM) prevented the decrease in TEER, and this effect was inhibited by the TRPV1 antagonist capsazepine (Fig. 7A). Apically, OEA also recovered the increased permeability when applied 24 h after cytokines, also inhibited by capsazepine (Fig. 7C).

As before, application of OEA to the basolateral membrane caused further decrease in TEER (when added at time 0 or 24 h), which was inhibited by the PPARα antagonist GW6471 but not by capsazepine (Fig. 7A, C).

As before, PEA (at the basolateral membrane) prevented the decline in TEER caused by cytokines when applied at the same time or 24 h later, and this PEA effect was inhibited by GW6471 (Fig. 7B, D).

### The effects of OEA and PEA on prolonged cytokine exposure

Last, we examined whether OEA and PEA can alter the permeability response to prolonged cytokine exposure. At 48 h after application of cytokines, apical application of OEA restored permeability to baseline (Fig. 8A). However, after 72 h of inflammation, this ability of OEA was lost (Fig. 8C). At the basolateral membrane, PEA restored permeability to baseline when applied 48 h after cytokine exposure (Fig. 8B) and even at 72 h after cytokine exposure (Fig. 8D), and this effect of PEA was inhibited by the PPARα antagonist GW6471 (Fig. 8D).

### DISCUSSION

This study has shown the effects of the endocannabinoid-like compounds OEA and PEA on the function and permeability of intestinal epithelial cells in control conditions and in inflammation. Both compounds reversed the hyperpermeability associated with inflammatory conditions through different mechanisms: OEA through TRPV1 on the apical membrane and PEA at the basolateral membrane.
through PPARα. Increased cellular and secreted OEA and PEA levels were observed in response to inflammation, suggesting that their local release plays a role in intestinal permeability. Inhibition of the degradation of these compounds augmented their responses, indicating that their effects are via the compounds themselves and not by their metabolites. It also suggests that the beneficial effects of these compounds could be augmented by coadministration of inhibitors of their degradation.

**OEA**

OEA production in the gut is stimulated by food intake (38) or reduced by food deprivation (39). It suppresses food intake, induces satiety, and decreases body weight gain (43) via PPARα activation (44). In intestinal epithelial cells, under control conditions, we found that apical administration of OEA increased Caco-2 monolayer resistance (i.e., decreased permeability) in a concentration-dependent manner via TRPV1. In contrast, OEA increased permeability when applied to the basolateral membrane by activation of TRPV1 and PPARα receptors. Although it is not known whether OEA stimulation by food intake would occur at the basolateral or apical membrane, based on the findings of the present study, alterations in permeability are likely to be associated.

Contractile filamentous actin networks regulate cellular shape change, which can be spatially and temporally modulated during physiologic processes such as cell adhesion, where cytoskeletal mechanics facilitate cell spreading and stiffening in response to environmental cues. F-actin structures, such as lamella and stress fibers, can facilitate adhesion, whereas cortical F-actin influences shape. FAK is a nonreceptor protein kinase that can modulate barrier function (54), and our data confirm that OEA transiently activates FAK. However, the F-actin changes that ensue are 2-fold. Apically, the cortical arrangement indicates shape change, whereas basally, the filamentous structure is reduced. Reduced adhesion at the base of the cells could explain why OEA has a differential effect on TEER, depending on where it is acting. It is unclear how the change in apical morphology connects to a change in cell–cell junctional complexes such that the interactions are tighter, but certainly reduced cellular adhesion to the extracellular matrix (or glass slide in this instance) could account for the OEA effect on permeability when applied basally. It is important to note that cells remain attached to each other with no gaps, implying that the changes in permeability are not related to pore formation or destruction of the epithelial monolayer (also indicated by the lack of effect of OEA on cell viability).

There are many molecular markers that are associated with barrier integrity and membrane permeability. Apical junctional complex structure can be dynamic and the precise location of some of the component parts can influence the final outcome. The contribution and mechanisms of AQPs in regulation of membrane permeability in the gut is unclear. In our study, the reduction in AQP4 membrane protein expression by OEA was unlikely to affect water transport, because knockdown of AQP4 has

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**Figure 5.** The effects of FAAH inhibition on OEA and PEA modulation of Caco-2 permeability. *A, C*) The effects of OEA applied to the apical (*A*) or basolateral (*C*) side of the Caco-2 monolayer alone and in the presence of the FAAH inhibitor (URB597, 1 μM), in combination with receptor antagonists. *B, D*) The effects of PEA applied to the apical side (*B*) or basolateral (*D*) side of the Caco2 monolayer alone or in the presence of URB597 in combination with receptor antagonists. Data are expressed as means ± SEM (n = 3).
no impact on the colonic osmotic water permeability coefficient (55, 56), but could be related to other functions, such as the intestinal inflammatory response (57). With regard to AQP3, apical expression in the ileum is reduced in early IBD (58), which may limit excessive water loss or alleviate oxidative stress. However, Zhang and colleagues (59) showed that intestinal barrier integrity is impaired by the knockdown of AQP3 by enhancement of paracellular permeability. OEA led to a modest reduction in expression of AQP3 in our study that would be unlikely to affect TEER through water transport. The role of these AQPs in glycerol and lipid metabolism is beyond the scope of this study, although it is tempting to speculate that the accepted contribution of OEA in fat sensing and transport of dietary lipids (60) could be mediated through AQP expression.

Figure 6. The effects of OEA and PEA in a model of increased permeability induced by cytokines. A, B) The effect of OEA and PEA (3 μM) on the permeability induced by cytokines (10 ng/ml) when applied apically and basolaterally at 0 h (A) or at 24 h (B), after the induction of inflammation. Data are expressed as means ± SEM (n = 3). *P < 0.01, **P < 0.01, ***P < 0.001, by 2-way repeated-measures ANOVA with post hoc analysis vs. vehicle control data. Solid horizontal bar: time of cytokine exposure; arrow: time point of application of OEA/PEA. C–F) Cells were grown to confluence in T75 flasks (n = 6 per condition) and exposed to the inflammation protocol. OEA (C, D) and PEA (E, F) levels were measured by mass spectrometry in the cellular lysate (C, E) or medium (D, F). Data are presented as means ± SEM. ***P < 0.001, ****P < 0.0001, by Student’s t test.
We also showed that OEA activated potassium channels in Caco-2 cells—an effect that has also been observed in arteries (61, 62). Potassium channel activation in the intestine is associated with many aspects of colonic epithelial function, including regulating electrogenic transport, regulating cell volume, and cellular migration (63, 64), suggesting OEA modulates epithelial cell functions in the intestine at many levels, which requires further investigation.

Regulation of the intercellular junctional interactions that maintain barrier function is highly complex. However, FAK activity through phosphorylation has been well correlated with TEER and Src dependency may be crucial to this function, particularly in Caco-2 cells (54). In our study, OEA transiently increased the autophosphorylation of FAK, but reduced Src phosphorylation in the same time frame. Reduced phosphorylation of Src and JNK have been shown to attenuate stretch-induced reorganization of the actin cytoskeleton (65), and increased Src is associated with tight junction disruption in the intestinal epithelium (66). The increase in p70S6K and CREB phosphorylation is likely to relate to downstream gene transcription and protein translation, which is similar to PEA. However, the increase in NF-κB activity, which is unique to OEA in this system, requires further investigation. NF-κB has pleiotropic roles in cell survival and the immune response. The precise role of NF-κB in TEER in this context is unclear, but may explain the basolateral reduction in TEER, reminiscent of TNFα-induced barrier disruption (67).

In our model of inflammation, IFNγ and TNFα applied to the basolateral membrane of confluent Caco-2 cells increased permeability, similar to that previously reported by our group (35). We found that application of OEA apically, concurrently with the cytokines, or even 24 or 48 h later, reversed the increased permeability via TRPV1. This is the first study to investigate the effects of OEA on intestinal permeability in vitro, but OEA has been found to decrease blood–brain barrier permeability in ischemia in vivo and in vitro similarly by PPARα activation (68). Pharmacological activation of TRPV1 may contribute to colonic inflammation (30); thus, the anti-inflammatory actions of OEA through TRPV1, may be brought about by desensitization of the TRPV1 receptor. We also showed that inflammation significantly increased OEA levels in Caco-2 cells, suggesting that these observations of the pharmacological effects of OEA have a physiologic relevance. Others have similarly shown that OEA is upregulated in response to inflammation (69) or by feeding (70), and this may be as a result of increased OEA synthesis or reduced degradation.

To summarize the effects of OEA, at the apical membrane OEA decreases permeability and inhibits increased permeability when applied before or after the induction of increased permeability associated with inflammation via TRPV1 activation, and inflammation increases cellular levels of OEA. By contrast, at the basolateral membrane, OEA causes increased permeability through both TRPV1 and PPARα. Activation of FAK, inactivation of Src, changes in F-actin, activation of K+ channels, and downregulation of AQP5 may underlie these cellular responses to OEA.

PEA

PEA is currently available as a nutraceutical food for medical purposes under the brand names Normast,
Pelvilen, and PeaPure; has been studied in humans, mostly within trials on pain management; and is well tolerated (71). Several preclinical animal studies have shown that in vivo treatment with PEA reduces intestinal injury and inflammation via PPARα (47, 48) and also CB2 and GPR55 (49). In support of this, we showed that PEA decreases Caco-2 cell permeability when applied to either the apical or basolateral membrane in a time- and concentration-dependent manner, also via activation of the PPARα receptor. Furthermore, basolateral application of PEA, as might occur with systemic administration, also reversed the hyperpermeability associated with inflammation via PPARα. Unlike with OEA, there was no negative (i.e., increased permeability) response to PEA application at either membrane. In inflammation, this beneficial effect of PEA was observed when PEA was added before the insult, or even after 24, 48, and 72 h after induction of inflammation. This ability of PEA to prevent increased permeability at the intestine barrier, via PPARα, is likely to underpin some of the beneficial effects seen in vivo. The increase in cellular PEA levels in response to our inflammatory protocol is in keeping with the proposed protective effects of endogenously produced PEA in the gut (49, 72, 73).

Like OEA, PEA also induced FAK activity, but the timing was more extended and the F-actin lamella structure seen with PEA was pronounced. These differences imply different cellular outcomes. The effect of PEA on the filament formation appears more typical, in that FAK phosphorylation resulted in F-actin polymerization and the filaments formed with focal adhesion plaques, both at the apical cell-to-cell contacts and the basement membrane (glass slide in our case), meaning that, functionally, these increases in turn increase cellular tension and adhesion to each other as well as to the “matrix”/adhesive surface, resulting in increased TEER, whether applied apically or basally. Like OEA, PEA also led to a modest but significant reduction in both AQP3 and -4 in the membrane fraction and activated potassium channels (see earlier paragraph). The role of FAK activity in terms of transient and prolonged phosphorylation could have an impact on the transient vs. sustained cytoskeletal changes in this study. However, the rather blunt tool of immunoblot analysis may distort the more subtle contribution of location and binding partners.

To summarize the effects of PEA at both the apical and basolateral membrane, PEA decreases permeability and inhibits increased permeability when applied before or up to 72 h after the induction of inflammation via PPARα, and inflammation increases cellular levels of PEA. Activation of FAK, inactivation of Src, changes in F-actin, activation of K⁺ channels, and down-regulation of AQP5 may underlie these cellular responses. PEA treatment is feasible and tolerated in humans and the present studies provide a potential rationale to justify controlled clinical trials of PEA in GI disorders.

CONCLUSIONS

OEA and PEA modulate intestinal permeability in normal and inflammatory conditions; OEA can both increase and
decrease permeability (via TRPV1) when applied to the apical or basolateral membrane, respectively, whereas PEA always decreases permeability i.e., increases resistance (via PPARs). Cellular levels of OEA and PEA are increased in intestinal epithelial cells in response to inflammation, which may limit the increased permeability associated with inflammation. The beneficial effects on intestinal permeability may at least partly underlie the protective effects of PEA on intestinal damage recently observed in preclinical studies. PPARs agonism, PEA administration or inhibiting PEA enzymatic degradation represent a novel range of therapeutic approaches against several intestinal disorders associated with increased intestinal permeability, including inflammatory bowel disease and the acute intestinal ischemia associated with circulatory shock.

ACKNOWLEDGMENTS

The authors thank Mrs. Averil Warren and Mr. Andy Lee (University of Nottingham) for technical assistance. The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

D. A. Barrett, K. L. Wright, M. Larvin, J. N. Lund, and S. E. O’Sullivan conceived of and designed the study; M. A. Karwad, T. Macpherson, B. Want, E. Theophilidou, S. Sarmad, K. L. Wright, and S. E. O’Sullivan generated, collected, assembled, analyzed, and interpreted the data; and all authors drafted or revised the manuscript and approved the final version.

REFERENCES

1. Helander, H. F., and Fändriks, L. (2014) Surface area of the digestive tract—revisited. Scand. J. Gastroenterol. 49, 681–689
2. Groschwitz, K. R., and Hogan, S. P. (2009) Intestinal barrier function: molecular regulation and disease pathogenesis. J. Allergy Clin. Immunol. 124, 3–20, quiz 21–22
3. Ulluwishewa, D., Anderson, R. C., McNabb, W. C., Moughan, P. J., Wells, J. M., and Roy, N. C. (2011) Regulation of tight junction permeability by intestinal bacteria and dietary components. J. Nutr. 141, 709–776
4. Turner, J. R. (2006) Molecular basis of epithelial barrier regulation: from basic mechanisms to clinical application. Am. J. Pathol. 169, 1901–1909
5. Turner, J. R. (2009) Intestinal mucosal barrier function in health and disease. Nat. Rev. Immunol. 9, 799–809
6. Brandtzæg, P. (2011) The gut as communicator between environment and host: immunological consequences. Eur. J. Immunol. 41(Suppl 1), S16–S32
7. Welcker, K., Martin, A., Kölle, P., Sieche, M., and Gross, M. (2004) Increased intestinal permeability in patients with inflammatory bowel disease. Eur. J. Med. Res. 9, 456–460
8. Sartor, R. B. (2006) Mechanisms of disease: pathogenesis of Crohn’s disease and ulcerative colitis. Nat. Clin. Pract. Gastroenterol. Hepatol. 3, 390–407
9. Mankertz, J., and Schulzke, J. D. (2007) Altered permeability in inflammatory bowel disease: pathophysiology and clinical implications. Curr. Opin. Gastroenterol. 23, 379–383
10. Hering, N. A., Fromm, M., and Schulzke, J. D. (2012) Determinants of colonic barrier function in inflammatory bowel disease and potential therapeutics. J. Physiol. 590, 1035–1044
11. Heyman, M., Abel, J., Lebretcon, C., and Cerf-Bensussan, N. (2012) Intestinal permeability in colitis disease: insight into mechanisms and relevance to pathogenesis. Gut 61, 1355–1364
12. Dunlop, S. P., Hebden, J., Campbell, E., Naesdal, J., Olbe, L., Perkins, A. C., and Spiller, R. C. (2006) Abnormal intestinal permeability in subgroups of diarrhea-predominant irritable bowel syndromes. Am. J. Gastroenterol. 101, 1288–1294
13. Camilleri, M., Lasch, K., and Zhou, W. (2012) Irritable bowel syndrome: methods, mechanisms, and pathophysiology: the confluence of increased permeability, inflammation, and pain in irritable bowel syndrome. Am. J. Physiol. Gastrointest. Liver Physiol. 303, G775–G785
14. Camilleri, M., Madsen, K., Spiller, R., Greenwood-Van Meerveld, B., and Verne, G. N. (2012) Intestinal barrier function in health and gastrointestinal disease. Neurogastroenterol. Motil. 24, 505–512
15. Bischoff, S. C. (2011) ‘Gut health’: a new objective in medicine? BMJ 343, d424
16. Hollander, D., Vadheim, C. M., Bretholz, E., Petersen, G. M., Delahunty, T., and Rotter, J. I. (1986) Increased intestinal permeability in patients with Crohn’s disease and their relatives: a possible etiologic factor. Ann. Intern. Med. 105, 883–885
17. May, G. R., Sutherland, L. R., and Meddings, J. B. (1993) Is small intestinal permeability really increased in relatives of patients with Crohn’s disease? Gastroenterology 104, 1627–1632
18. Wyatt, J., Oberhuber, G., Pongratz, S., Piasnik, A., Moser, G., Novack, G., Locks, H., and Vogelsang, H. (1997) Increased gastric and intestinal permeability in patients with Crohn’s disease. Am. J. Gastroenterol. 92, 1891–1896
19. Odenwald, M. A., and Turner, J. R. (2013) Intestinal permeability defects: is it time to treat? Clin. Gastroenterol. Hepatol. 11, 1075–1083
20. Mechoulam, R. (1986) The Pharmacohistory of Cannabis sativa. CRC Press, Boca Raton, FL, USA
21. Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N. E., Schatz, A. R., Gopher, A., Almog, S., Martin, B. R., Compton, D. R., Pertwee, R. G., Griffin, G., Bayewitch, M., Barg, J., and Vogel, Z. (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem. Pharmacol. 50, 85–90
22. Van Sickle, M. D., Oland, L. D., Ho, W., Hillard, C. J., Mackie, K., Davison, J. S., and Starkey, K. A. (2001) Cannabinoids inhibit emesis through CB1 receptors in the brainstem of the ferret. Gastroenterology 121, 767–774
23. Izzo, A. A., Pezza, F., Capasso, R., Bisogno, T., Pinto, L., Inovne, T., Esposito, G., Mascolo, N., Di Marzo, V., and Capasso, F. (2001) Cannabinoid CB1-receptor mediated regulation of gastrointestinal motility in mice in a model of intestinal inflammation. Br. J. Pharmacol. 134, 563–570
24. Adami, M., Frati, P., Bertini, S., Kulkarni-Narla, A., Brown, D. R., de Caro, G., Coruzzi, G., and Solandini, G. (2002) Gastric antisecretory role and immunohistochemical localization of cannabinoid receptors in the rat stomach. Br. J. Pharmacol. 135, 1598–1606
25. Maccarrone, M., and Finazzi-Agrò, A. (2003) The endocannabinoid system, anandamide and the regulation of mammalian cell apoptosis. Cell Death Differ. 10, 946–955
26. Klein, T. W. (2005) Cannabinoid-based drugs as anti-inflammatory therapeutics. Nat. Rev. Neurosci. 6, 400–411
27. Di Marzo, V., and Izzo, A. A. (2006) Endocannabinoid overactivity and intestinal inflammation. Gut 55, 1373–1376
28. Di Carlo, G., and Izzo, A. A. (2005) Cannabinoids for gastrointestinal diseases: potential therapeutic applications. Expert Opin. Investig. Drugs 12, 39–40
29. Pertwee, R. G. (1997) Pharmacology of cannabinoid CB1 and CB2 receptors. Pharmacol. Ther. 74, 129–180
30. Massa, F., Sibau, A., Massari, G., Blaudzun, H., Storr, M., and Lutz, B. (2006) Vanilloid receptor (TRPV1)-deficient mice show increased susceptibility to dinitrobenzene sulfonic acid induced colitis. J. Mol. Med. 94, 142–146
31. O’Sullivan, S. E. (2007) Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. Br. J. Pharmacol. 152, 578–582
32. Fredriksson, R., Höglund, P. J., Glimelius, D. E., Lagerström, M. C., and Glöer, P. J. (2007) The orphan receptor GPR55 is a novel cannabinoid receptor. Br. J. Pharmacol. 151, 1421–1429
33. Allegri, A., Lee, A. C., Wright, K. L., Larvin, M., and O’Sullivan, S. E. (2010) Pharmacological effects of cannabinoids on the Caco-2
cell culture model of intestinal permeability. J. Pharmacol. Exp. Ther. 335, 92–102
35. Alhamaruni, A., Wright, K. L., Larvin, M., and O’Sullivan, S. E. (2012) Cannabinoids mediate opposing effects on inflammation-induced intestinal permeability. Br. J. Pharmacol. 165, 2598–2610
36. Murugan, G. B., Nasdali, D., Bicknell, M. S., Lambert, D. M., Delzenne, N. M., and Cani, P. D. (2010) The endocannabinoid system links gut microbiota to adipogenesis. Mol. Syst. Biol. 6, 392
37. Piomelli, D., Beltramino, M., Giuffrida, A., and Stella, N. (1998) Endogenous cannabinoid signaling. Neurobiol. Dis. 5, 462–473
38. Astari, G., Rourke, B. C., Andersen, J. B., Fu, J., Kim, J. H., Bennett, A. F., Hicks, J. W., and Piomelli, D. (2006) Postprandial increase of oleoylethanolamide mobilization in small intestine of the Burmese python (Python molurus). Am. J. Physiol. Regul. Integr. Comp. Physiol. 290, R1407–R1412
39. Izzo, A. A., Piscitti, F., Capasso, R., Marini, P., Cristino, L., Petrosino, S., and Di Marzo, V. (2010) Basal and fasting/refeeding-regulated tissue levels of endogenous PPAR-alpha ligands in Zucker rats. Obesity (Silver Spring) 18, 55–62
40. Fu, J., Gaetani, S., Oveisi, F., Lo Verme, J., Serrano, A., Rodriguez De Fonseca, F., Rosengarth, A., Luccke, H., Di Giacomo, B., Tarzia, G., and Piomelli, D. (2005) Oleoylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPARalpha. Nature 435, 90–93
41. Ahern, G. P. (2003) Activation of TRPV1 by the satiety factor oleoylethanolamide. J. Biol. Chem. 278, 30429–30434
42. Borrelli, F., and Izzo, A. A. (2009) Role of acylethanolamides in the gastrointestinal tract with special reference to food intake and energy balance. Best Pract. Res. Clin. Endocrinol. Metab. 23, 33–49
43. Rodriguez de Fonseca, F., Navarro, M., Gomez, R., Escuredo, L., Nava, F., Fu, J., Murillo-Rodriguez, E., Giuffrida, A., LoVerme, J., Gaetani, S., Kadhuria, S., Gall, C., and Piomelli, D. (2001) An anorexix lipid mediator regulated by feeding. Nature 411, 209–212
44. LoVerme, J., Gaetani, S., Fu, J., Oveisi, F., Burton, K., and Piomelli, D. (2005) Regulation of food intake by oleoylethanolamide. Cell. Mol. Life Sci. 62, 708–716
45. Guzmán, M., LoVerme, J., Fu, J., Oveisi, F., Blázquez, C., and Piomelli, D. (2004) Oleoylethanolamide stimulates lipolysis by activating the nuclear receptor peroxisome proliferator-activated receptor alpha (PPARalpha). J. Biol. Chem. 279, 27849–27854
46. Fu, J., Oveisi, F., Gaetani, S., Lin, E., and Piomelli, D. (2005) Oleoylethanolamide, an endogenous PPAR-alpha agonist, lowers body weight and hyperlipidemia in obese rats. Neuropharmacology 48, 1147–1153
47. Esposito, G., Capoccia, E., Turco, F., Palumbo, I., Lu, J., Steardo, A., Xu, Z., and Xu, H. (2011) Knockdown of PPAR-alpha inhibits expression of aquaporin-4 in the colon of streptozotocin-induced diabetic mice. Biochim. Biophys. Acta 1812, 151–159
48. Di Marzo, V., and Izzo, A. A. (2014) Palmitoylethanolamide normalizes intestinal permeability. Br. J. Pharmacol. 171, 458–467
49. Leotta, C. (2014) Fatty gut feeding. Trends Endocrinol. Metab. 25, 332–341
50. Wheal, A. J., Alexander, S. P., and Randall, M. D. (2010) Vasorelaxation to N-oleoylethanolamine in rat isolated arteries: mechanisms of action and modulation via cyclooxygenase activity. Br. J. Pharmacol. 160, 701–711
51. Alduizenma, Y. M., and Hiley, C. R. (2013) Mechanisms of vasorelaxation induced by oleoylethanolamide in the rat small mesenteric artery. Eur. J. Pharmacol. 702, 1–11
52. Rao, J. N., Platschyn, O., Li, L., Guo, X., Golovina, V. A., Yuan, J. X., and Wang, J. Y. (2002) Activation of K+ channels and increased migration of differentiated intestinal epithelial cells after wounding. Am. J. Physiol. Cell Physiol. 282, C885–C898
53. Heitzman, D., and Wardh, R. (2008) Physiology and pathophysiology of potassium channels in gastrointestinal epithelia. Physiol. Rev. 88, 1119–1182
54. Samak, G., Gangwar, R., Crosby, L. M., Desai, L. P., Wilhelm, K., Waters, C. M., and Rao, R. (2014) Cyclic stretch disrupts apical junctional complexes in Caco-2 cell monolayers by a JIP2-, c-Src- and MLCK-dependent mechanism. Am. J. Physiol. Gastrointest. Liver Physiol. 306, C847–C858
55. Basuroy, S., Sheeth, P., Kappusswamy, D., Balasubramanian, S., Rav, R. M., and Rao, R. K. (2003) Expression of kinase-inactive c-Src delays oxidative stress-induced disassembly and accelerates calcium-mediated reassembly of tight junctions in the Caco-2 cell monolayer. J. Biol. Chem. 278, 11916–11924
56. Ma, T. Y., Iwamoto, G. K., Hoa, N. T., Akotia, V., Pedram, A., Boivin, M. A., and Said, H. M. (2004) TNF-alpha-induced increase in intestinal epithelial tight junction permeability requires NF-kappa B activation. Am. J. Physiol. Gastrointest. Liver Physiol. 286, G307–G316
57. Zhou, Y., Yang, L., Ma, A., Zhang, X., Li, W., Yang, W., Chen, C., and Jin, X. (2012) Orally administered oleoylethanolamide protects mice from focal cerebral ischemic injury by activating peroxisome proliferator-activated receptor gamma. Neuropharmacology 63, 224–243
58. Schillitii, C., Cacinottia, L., Fedele, V., Ingegnosi, C., Luca, S., and Leotta, C. (2014) Micronized palmitoylethanolamide reduces the symptoms of neuropathic pain in diabetic patients. [E-pub ahead of print] Pain Res. Treat. 2011, 849623
59. Fu, J., Astari, G., Gaetani, S., Kim, J., Cravatt, B. F., Mackie, K., and Piomelli, D. (2007) Food intake regulated by oleoylethanolamide formation and degradation in the proximal small intestine. J. Biol. Chem. 282, 1518–1528
60. Schillitii, C., Cacinottia, L., Fedele, V., Ingegnosi, C., Savoca, G., and Leotta, C. (2014) Palmitoylethanolamide reduces the symptoms of neuropathic pain in diabetic patients. Pain Res. Treat. 2014, G849623
61. Wang, J., Zheng, J., Kulkarni, A., Wang, W., Garg, S., Prather, P. L., and Hauer-Jensen, M. (2014) Palmitoylethanolamide regulates development of intestinal radiation injury in a mast cell-dependent manner. Dig. Dis. Sci. 59, 2703–2710
62. Capasso, R., Orlando, P., Capoano, T., Di Marzo, V., and Izzo, A. A. (2014) Palmitoylethanolamide normalizes intestinal motility in a model of postinflammatory accelerated transit: involvement of CB receptors and TRPV1 channels. Br. J. Pharmacol. 171, 4586–4597

Received for publication August 9, 2016. Accepted for publication August 15, 2016.