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ORIGINAL RESEARCH—BASIC

Cannabinoids Block Fat-induced Incretin Release via CB_1-dependent and CB_1-independent Pathways in Intestinal Epithelium

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BACKGROUND AND AIMS: Glucose homeostasis is regulated by a dynamic interplay between hormones along the gastroinsular axis. For example, enteroendocrine L- and K- cells that line the intestine produce the incretins glucagon-like peptide-1 (GLP1) and glucose-dependent insulinotropic polypeptide (GIP), respectively, which are secreted following a meal. Broadly, incretin signaling enhances insulin release from the endocrine pancreas and participates in the control of food intake, and therapeutics that mimic their activity have recently been developed for the treatment of type-2 diabetes and obesity. Notably, genes for cannabinoid subtype-1 receptor (CB_1R) are expressed in these cell subpopulations; however, roles for CB_1Rs in controlling fat-induced incretin release are unclear. To address this gap in our understanding, we tested the hypothesis that intestinal epithelial CB_1Rs control fat-induced incretin secretion. **METHODS:** We treated mice with conditional deletion of CB_1Rs in the intestinal epithelium ($IntCB_1-/-$) or controls ($IntCB_1+/+$) with oil gavage to stimulate incretin release in the presence of the cannabinoid receptor agonists, WIN55,212-2 or Δ^9 tetrahydrocannabinol (THC), and the peripherally-restricted CB_1R antagonist AM6545. Circulating incretin levels were measured in plasma. **RESULTS:** Oral gavage of corn oil increased levels of bioactive GLP1 and GIP in $IntCB_1+/+$ mouse plasma. Pretreatment with the WIN55,212-2 or THC blocked this response, which was largely reversed by coadministration with AM6545. WIN55,212-2 failed to inhibit fat-induced GIP release, but not GLP1, in $IntCB_1-/-$ mice. In contrast, THC inhibited the secretion of incretins irrespective of CB_1R expression in intestinal epithelial cells. **CONCLUSION:** These results indicate that cannabinoid receptor agonists can differentially inhibit incretin release via mechanisms that include intestinal epithelial CB_1R-dependent and CB_1R-independent mechanisms.

Keywords: Incretin; Cannabinoid; Intestinal Epithelium

Introduction

The endocannabinoid system controls food intake, energy balance, and glucose homeostasis.[1–6] For example, studies from our group and others indicate that central and peripheral cannabinoid subtype-1 receptor (CB_1R) signaling promotes the intake of palatable foods.[5,7–12] CB_1Rs are expressed on enteroendocrine I-cells, which produce and secrete the satiation peptide cholecystokinin (CCK).[13] During diet-induced obesity, increased levels of endocannabinoids in the proximal small intestinal epithelium activate CB_1Rs and inhibit the secretion of CCK, which results in hyperphagia.[14] Furthermore, intestinal epithelial CB_1Rs control acute dietary preference for highly palatable foods.[15] CB_1R mRNA is also expressed in enteroendocrine L- and K-cells, which produce the incretins glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide (GLP1 and GIP, respectively).[16,17] These incretin hormones activate their respective receptors along the gastropancreatic axis to enhance insulin secretion and are responsible for 50%–70% of all postprandial insulin secretion.[18] Limited studies have suggested a role for CB_1Rs in incretin release; however, identification of specific roles that CB_1Rs in the intestinal epithelium play – and the impact of exogenous cannabinoids on these processes – requires further research.[16,19]

The incretin receptors are pharmacological targets for new-generation therapeutics to treat type-2 diabetes mellitus, and more recently, obesity.[20] Indeed, GLP1 mimetics (eg, semaglutide, liraglutide) reduce body mass in obese and diabetic patients, partly due to the hypophagic effects of activating GLP1 receptors.[21] Interestingly, recent results also suggest that dual GLP1/GIP receptor agonists

Abbreviations used in this paper: **aGLP1, active glucagon-like polypeptide 1; AM, AM6545; CB_1R, Cannabinoid receptor subtype-1; CB_2R, Cannabinoid receptor subtype-2; CCK, cholecystokinin; CO, Corn oil; DPPIV, dipeptidyl peptidase 4; FFA, free fatty acid; GIP, glucose-dependent insulinotropic polypeptide; GLP1, glucagon-like polypeptide 1; GPR55, G-protein coupled receptor 55; $IntCB_1+/+$, wildtype control mice; $IntCB_1-/-$, mice lacking intestinal CB_1R; THC, Δ^9 tetrahydrocannabinol; WIN, WIN 55,212-2.**

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(eg, tirzepatide) may be more efficacious at reducing body weight and lowering hemoglobin A1c, when compared to GLP1 receptor agonists alone.[20,22] Accordingly, it will be important to identify the cellular and molecular mechanisms that may differentially control the release of incretins, which will be important to inform the development of safe and effective treatment options for type-2 diabetes and obesity.

Recreational use of cannabis has steadily increased in the United States[23] over the past several decades. Cannabis contains a variety of bioactive compounds, including Δ^9 tetrahydrocannabinol (THC), and has recently been studied for its possible medicinal applications in metabolic diseases. Interestingly, acute cannabis use is correlated with increases in food intake[24,25]; however, chronic consumption of cannabis is paradoxically associated with lower rates of obesity and reduced probability of developing type-2 diabetes in humans.[26–34] Cannabinoid receptors regulate food intake and may control energy and glucose homeostasis through metabolic hormones such as incretins. Thus, dysregulated cannabinoid receptor control of incretin hormones may contribute to type-2 diabetes and obesity. Here, we investigated the effects of exogenous activation of peripheral and intestinal epithelial CB_1Rs in the control of fat-induced GLP1 and GIP secretion.

Materials and Methods

Animals

Male 8–12 week-old C57BL/6 mice (Wildtype; Taconic, Oxnard, CA, USA), C57BL/6-Tg (Vil-CreERT/1Lphi/J-Cnr1$^{tm1.1mll}$) (Int$CB_1-/-$), or littermate control (Int$CB_1+/+$) mice were used for these studies. Mice were maintained on a 12 hours dark/light cycle (lights off 6 pm), and group-housed with *ad libitum* food and water access (see Avalos et al[15] for generation of Int$CB_1-/-$). All procedures met the U.S. National Institute of Health guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of the University of California, Riverside.

Chemicals and Administration Schedule

The cannabinoid receptor agonist, WIN 55,212-2, was given by IP injection at 3 mg per kg (Cayman Chemicals, Ann Arbor, MI). AM6545, a peripherally-restricted CB_1R neutral antagonist, was given by IP injection at 10 mg per kg (Northeastern University Center for Drug Discovery, Boston, MA, USA). Both drugs were dissolved in a vehicle consisting of 7.5% DMSO, 7.5% Tween80, and 85% sterile saline, and warmed in a water bath to ensure solubility. Δ^9-tetrahydrocannabinol (THC, supplied by National Institutes of Drug Abuse) was given by IP injection at 5 mg per kg dissolved in 5% EtOH, 5% Tween80, and 90% sterile saline. Tamoxifen (10 mg per mL) was dissolved in corn oil, sonicated, and warmed in a water bath to ensure solubility, and stored away from light until use. Tamoxifen was given by IP injection at 40 mg per kg for 5 consecutive days to both Int$CB_1-/-$ and Int$CB_1+/+$ control mice.

Stimulation of Incretin Release and Hormone Quantification

Mice were acclimated to cages fitted with elevated wire bottoms for 72 hours to prevent coprophagia and fasted for 12 hours to ensure an empty stomach. Mice were pretreated with CB_1R ligands, then administered corn oil (0.5 mL) by oral gavage 30 minutes later. Blood was collected via cardiac puncture 30 minutes following gavage and was placed in BD vacutainer lavender top EDTA blood collection tubes on ice. Small intestine was quickly removed and washed in phosphate-buffered saline (PBS), opened longitudinally on a stainless-steel tray on ice, and contents removed. Intestinal epithelium of different regions was isolated using glass slides to scrape the epithelial layer and was snap-frozen in liquid N_2. Tissue samples were stored at −80 °C pending analysis. Blood plasma was obtained by centrifugation of tubes at 1500 G for 10 minutes at 4 °C. Hormone levels were quantified by a sensitive and selective GIP ELISA (EMD Millipore, St. Louis, MI, USA), active GLP1 ELISA (ALPCO, Salem, NH, USA), and insulin ELISA (EMD Millipore, St. Louis, MI, USA). GIP and Insulin ELISA reactions were measured using iMark microplate reader (BioRad, Hercules, CA, USA) and active GLP1 ELISA reactions were measured using Luminex MagPix instrument.

Gene Expression Analysis

RNA isolation. Total RNA was extracted from tissues using RNeasy kit (Qiagen, Valencia, CA, USA) method, and first-strand complementary DNA was generated using M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA). All surfaces for tissue collection and processing were sanitized using 70% ethanol and then treated with an RNase inhibitor (RNase out, G-Biosciences, St. Louis, MO, USA) to maintain the integrity of isolated RNA. Reverse transcription of total RNA (0.2–1.0 μg, tissue-specific) was performed as previously described.[35]

Quantitative polymerase chain reaction analysis. RT-qPCR was carried out using PrimePCR SYBR Green Assays (Biorad, Hercules, CA, USA) with the following primers for mouse genes: cannabinoid receptor subtype 1 (*Cnr1*), Neurogenin 3, glucose-dependent insulinotropic polypeptide (*GIP*), proglucagon, CCK, cluster of differentiation 36, and free fatty acid receptors 1–4 (*Ffar1-4*), *Hprt* was used as housekeeping gene. Values are expressed as relative mRNA expression using the delta-delta cq method.[36] Reactions were run in duplicate for each animal. Analysis of gene expression was performed on intestinal epithelium from mice used in our previously published work (see Ref. 15). We present expression levels of *Cnr1* in the current publication (Figure A1), which was previously published by us (see Ref. 15), to validate the knockdown of *Cnr1* in this tissue; however, all other data presented

in Figure A1 – aside from *Cnr1* – has not been previously published and is new data in this current publication.

Gastric Emptying

To evaluate drug effects on gastric emptying, corn oil was spiked with 2.5 nmol 19:2 FFA and administered by oral gavage (0.5 mL), then quantities of 19:2 FFA remaining in the stomach were evaluated at the time of blood collection 30 minutes after gavage. The stomach was removed and immediately placed into methanol containing 17:1 FFA as an internal standard. Lipids were extracted and 19:2 FFA was analyzed via UPLC/MS/MS as previously described.[14] Data were acquired using an Acquity I Class UPLC with direct connection to a Xevo TQ-S Micro Mass Spectrometer (Waters Corporation, Milford, MA, USA) with electrospray ionization (ESI) sample delivery. Lipids were separated using an Acquity UPLC BEH C_{18} column (2.1 $\times$ 50 mm i.d., 1.7 μm, Waters Corporation) and inline Acquity guard column (UPLC BEH C_{18} VanGuard PreColumn; 2.1 $\times$ 5 mm i.d.; 1.7 μm, Waters Corporation), and eluted by a gradient of water and methanol (containing 0.25% acetic acid, 5 mM ammonium acetate) at a flow rate of 0.4 mL per minute and gradient: 90% methanol 0.1 minutes, 90%–100% methanol 0.1–2.0 minutes, 100% methanol 2.0–2.1 minutes, 100%–90% methanol 2.1–2.2 minutes, and 90% methanol 2.2–2.5 minutes. Column was maintained at 40 °C and samples were kept at 10 °C in sample manager. MS detection was in negative ion mode with capillary voltage maintained at 3.00 kV. Cone voltages for nonadecadienoic acid (19:2 FFA) = 48 V and heptadecanoic acid (17:1 FFA) = 64 V. Lipids were quantified using a stable isotope dilution method of proton adducts of the molecular ions $[M - H]^-$ in selected ion recording (SIR) mode. Tissue processing and LCMS analyses for experiments occurred independently of other experiments. Extracted ion chromatograms for SIR masses were used to quantify analytes: 19:2 FFA (m/z = 293.2) and 17:1 FFA (m/z = 267.2) as internal standard.

DPPIV Enzyme Activity Assay

DPPIV enzymatic activity was determined in isolated plasma from control and intCB_1−/− mice by measuring the conversion of glycin-prolin-p-nitroanilide to p-nitroanilide. Twenty μL of plasma (in duplicate) was obtained from each subject and placed with 90 μL assay buffer (50 mM glycine, 1.0 mM EDTA, pH 8.7) and 10 μL glycine-proline-p-nitroanilide (5 mM). P-nitroanilide was measured in kinetic measurement at 37C (405 nm) over 30 minutes and enzymatic activity was calculated against a standard curve (100 μM–2.0 mM). Data expressed as nmol/min/mL.[37]

Institutional Review Board Statement

This animal study was approved by the University of California Riverside Institutional Animal Care and Use Committee (IACUC) (protocol A-20200022, and #9) and was in accordance with National Institutes of Health guidelines, the Animal Welfare Act, and Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Statistical Analysis

Values are expressed as means ± SEM. Unpaired Student's two-tailed t-test was used to compare data for gene analysis in different tissues between control and intCB_1−/− mice. One-way ANOVA was used to determine differences in multiple groups with post hoc Holm-Sidak's multiple comparisons tests. Two-way ANOVA was used to determine differences between 2 groups with post hoc Holm-Sidak's multiple comparisons tests. Additionally, repeated measures two-way ANOVA was used for groups measured over time. Data were analyzed using GraphPad Prism10.1.1 software. Significance was determined as $P < .05$. Statistical outliers were determined using Grubb's test in all data sets.

Results

Peripheral CB_1Rs Control Corn Oil-induced Release of Incretins

We previously reported that CB_1Rs are enriched in enteroendocrine I-cells and regulate the secretion of the satiation hormone, CCK.[14] In addition, *Cnr1* is expressed in other enteroendocrine populations, including K- and L-cells that produce and secrete GIP and GLP1.[16,38] Here, we investigated a role for CB_1Rs in the intestinal epithelium in controlling fat-induced secretion of the incretin hormones, GIP and GLP1. Oral gavage of mice with corn oil (CO) potently increased plasma levels of GIP and bioactive GLP1 (aGLP1) (Figure 1A and B). CO did not significantly affect circulating insulin levels (Figure 1C) when compared to control fasted mice that received oral gavage of saline. Administration of the cannabinoid receptor agonist, WIN 55,212-2 (WIN, 3 mg per kg), blocked CO-induced increases in levels of GIP and aGLP1 in the blood (Figure 1A and B). In addition, the inhibitory effects of WIN on CO-induced secretion of GIP and aGLP1 were reversed by cotreatment with the peripherally-restricted CB_1R antagonist, AM6545 (10 mg per kg) (Figure 1A and B). These results suggest that activation of CB_1Rs in the periphery with a synthetic cannabinoid inhibits fat-induced incretin secretion from the intestines.

THC Inhibits the Secretion of Incretins via Peripheral CB_1Rs

Next, we investigated the effects of the intoxicating chemical in cannabis, THC, on CO-induced GIP and aGLP1 secretion and roles for peripheral CB_1Rs in this response. Administration of THC (IP, 3 mg per kg) blocked CO-induced secretion of GIP and aGLP1 (Figure 2A and B). This effect was reversed by cotreatment with AM6545 (Figure 2A and B). We next evaluated gastric emptying following administration of THC and AM6545 to evaluate if changes (ie, reductions) in intestinal exposure to stomach contents may underlie some of

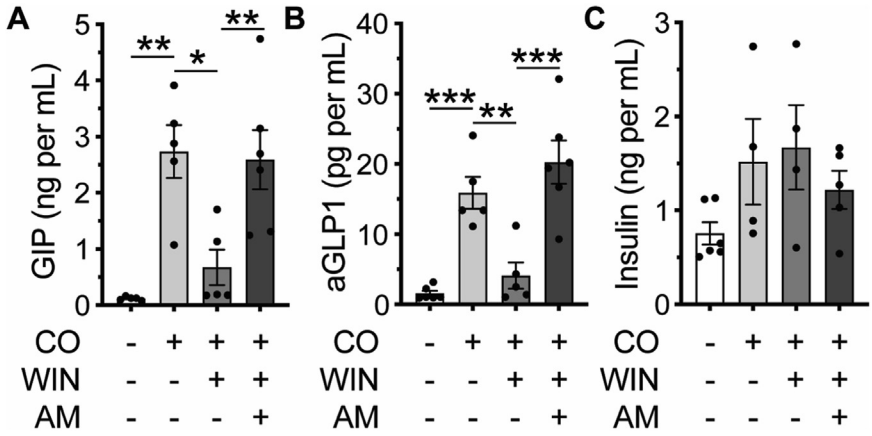

Figure 1. Exogenous activation of peripheral CB_1Rs blocks fat-induced GIP and aGLP1 secretion. Compared to the control (0.5 mL saline by oral gavage and vehicle by IP injection), corn oil (CO; 0.5 mL by oral gavage) increased levels of the incretins GIP [treatment effect $F_{(3,17)} = 10.53$, $P = .0004$] (A) and active GLP1 [treatment effect $F_{(3,17)} = 18.06$, $P < .0001$] (B) but did not significantly increase levels of plasma insulin (C) in plasma of fasted mice. This effect was blocked by pretreatment with CB_1R agonist, WIN 55,212-2 (WIN, IP 3 mg per kg 30 minutes before CO). The inhibitory effects of WIN on incretins were inhibited by coadministration with the peripherally-restricted CB_1R antagonist, AM6545 (AM; IP 10 mg per kg 30 minutes before CO). Data expressed as means ± S.E.M. and analyzed by one-way ANOVA with post hoc Holm-Sidak multiple comparison tests. n = 4–6 per condition, $^*P < .05$, $^{**}P < .01$, $^{***}P < .001$.

the decreased fat-induced incretin release. We previously reported that WIN and AM6545 did not inhibit gastric emptying (see Argueta et al,[14] 2019, Figure S4). Similarly, THC not only failed to inhibit gastric emptying, but rather lowered levels in the stomach of the 19:2 free fatty acid (FFA) tracer that was added to CO, which indicates a slight increase in gastric emptying (Figure 2C). Similar to our previously reported results for WIN treatment (see Argueta et al 2019,[14] Figure S5), THC did not affect blood glucose levels following gavage with CO (Figure 2D and E).

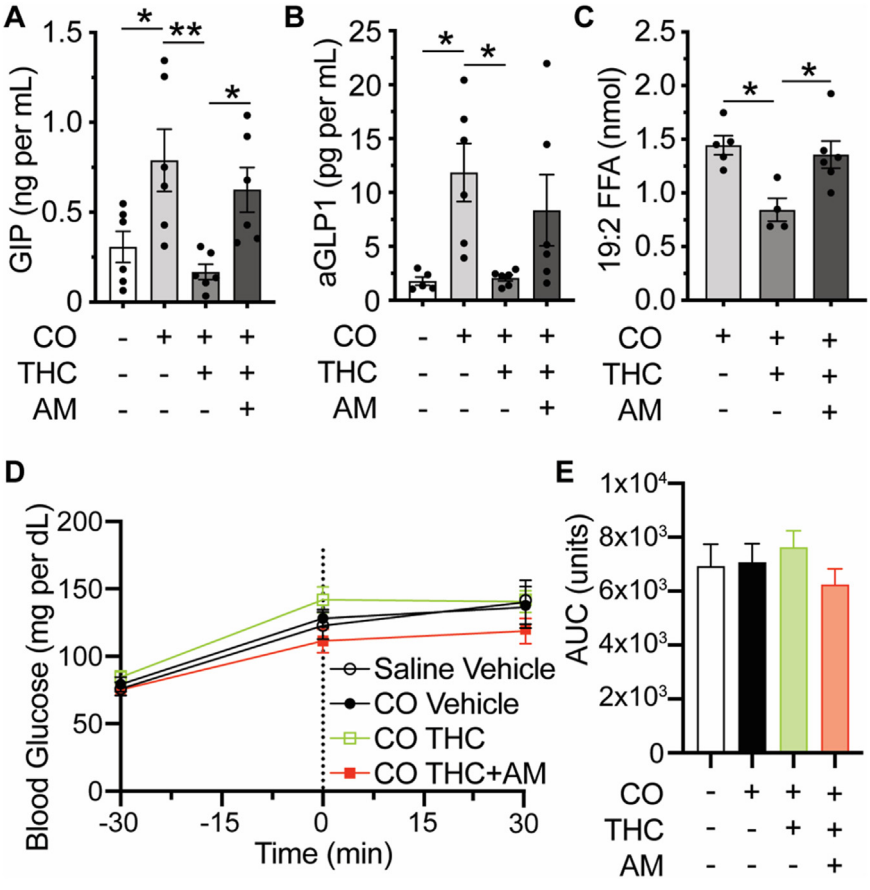

Figure 2. THC inhibits incretin secretion through peripheral CB_1Rs. As expected, CO increased the levels of plasma GIP [treatment effect $F_{(3,20)} = 5.943$, $P = .0045$] (A) and aGLP1 [treatment effect $F_{(3,19)} = 4.829$, $P = .0116$] (B) of fasted mice. This effect was blocked by pretreatment with cannabinoid receptor agonist THC (IP 3 mg per kg 30 minutes before CO). The inhibitory effects of THC on incretin release were inhibited by coadministration with AM. Gastric emptying results suggest an increase in gastric emptying under THC treatment alone, indicating more – not less – oil was emptied into the intestines [treatment effect $F_{(2,12)} = 7.121$, $P = .0091$] (C). Lastly, blood glucose levels throughout the experiment remained similar between conditions (D and E). Data expressed as means ± S.E.M. and analyzed by one-way ANOVA with post hoc Holm-Sidak multiple comparison test (A–C, and E) or by two-way ANOVA with repeated measures (D). n = 4–6 per condition, $^*P < .05$, $^{**}P < .01$.

CB_1Rs in the Intestinal Epithelium Differentially Control Incretin Release

We previously reported a role for CB_1Rs in the intestinal epithelium in governing acute preference for highly palatable foods,[15] maintaining gut barrier function during diet-induced obesity,[39] and anxiety-like behaviors.[40] Here, we tested the hypothesis that CB_1Rs in the intestinal epithelium mediate the inhibitory actions of cannabinoid receptor agonists on CO-induced GIP and aGLP1 secretion. As expected, CO stimulated the secretion of GIP and aGLP1 in $IntCB_1+/+$ control mice, which was blunted by treatment with WIN. In contrast to control mice, WIN failed to inhibit CO-induced GIP secretion in $IntCB_1-/-$ mice; however, WIN was able to block the secretion of aGLP1 in these mice (Figure 3A and B). CO did not significantly affect insulin levels in $IntCB_1-/-$ mice or $IntCB_1+/+$ control mice (Figure 3C). WIN treatment did not affect blood glucose levels (Figure 3D and E) or gastric emptying (Figure 3F), irrespective of genotype (ie, $IntCB_1-/-$ or $IntCB_1+/+$ mice). Together, these data suggest that CB_1Rs in the intestinal epithelium are required for reductions in lipid-induced GIP secretion following WIN treatment, but not GLP1.

THC Inhibits the Secretion of Incretins via an Intestinal Epithelial CB_1R-independent Mechanism

We next tested the effects of THC administration on GIP and aGLP1 release in $IntCB_1-/-$ and $IntCB_1+/+$ mice. As expected, THC blocked CO-induced GIP and aGLP1 secretion in $IntCB_1+/+$ control mice. Surprisingly, THC similarly inhibited fat-induced incretin release in $IntCB_1-/-$ mice, which suggests that THC may block incretin secretion through a mechanism that does not require CB_1Rs in the intestinal epithelium. (Figure 4A and B). THC treatment failed to significantly affect gastric emptying (Figure 4C) or blood glucose levels (Figure 4D and E), irrespective of genotype.

Expression of Genes for Incretins and Fat-sensing Proteins

Next, we analyzed the expression of genes in the intestinal epithelium that encode for proteins involved in the synthesis of the incretins as well as fat-sensing between $IntCB_1-/-$ and $IntCB_1+/+$ control mice (Figure A1). Expression of Neurogenin 3 – an important gene in directing

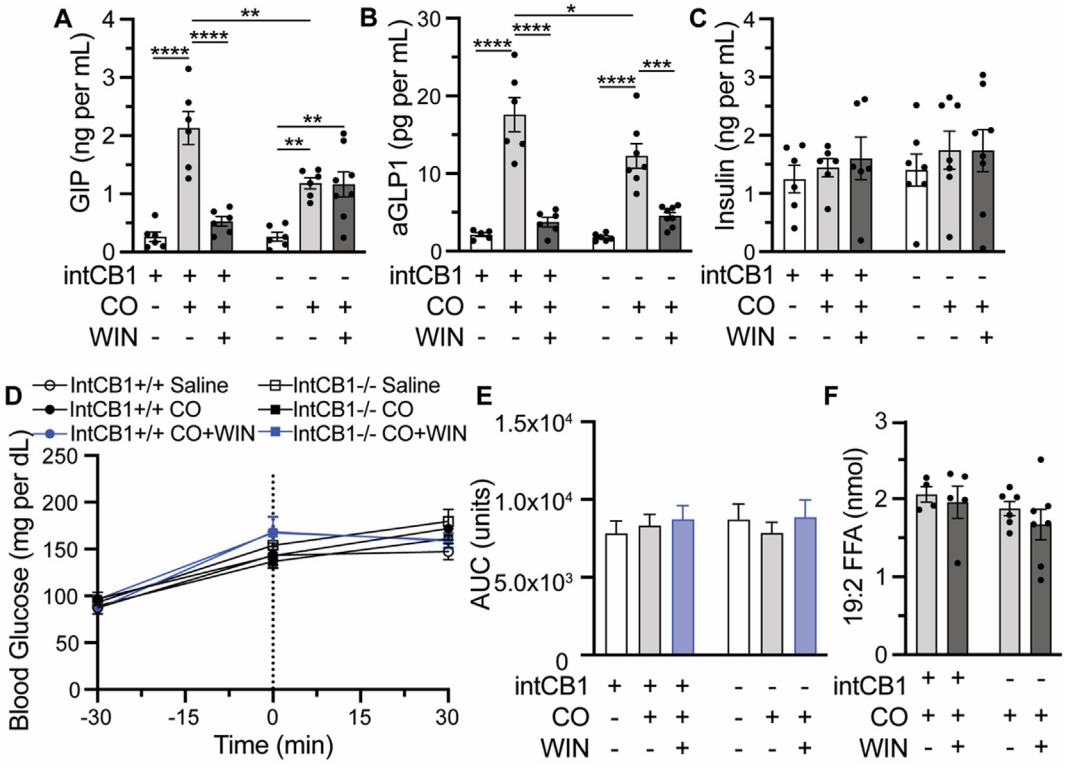

Figure 3. Intestinal epithelial CB_1Rs differentially control incretin secretion. As previously shown, CO greatly stimulates GIP release into circulation, an effect that is blunted with WIN pretreatment. However, $intCB_1-/-$ secrete GIP following CO gavage regardless of WIN pretreatment [treatment main effect $F_{(2,32)} = 11.00$, $P = .0002$; Interaction effect $F_{(2,32)} = 32.05$, $P = .0002$] (A). In addition, pretreatment with WIN blocks fat-induced aGLP1 secretion from $intCB-/-$ mice [treatment main effect $F_{(2,32)} = 69.57$, $P < .0001$; Interaction effect $F_{(2,32)} = 32.05$, $P = .0287$] (B). Plasma insulin levels remain unchanged following CO or WIN treatment and are unchanged in mice lacking intestinal epithelium CB_1Rs (C). Lastly, blood glucose levels (D), AUC of glucose timecourse (E), or gastric emptying of CO (F) remained similar between conditions. Data expressed as means ± S.E.M and analyzed by two-way ANOVA with post hoc Holm-Sidak multiple comparison test (A–C, E, and F) or by two-way ANOVA with repeated measures (D). n = 6–8 per condition, $^*P < .05$, $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$.

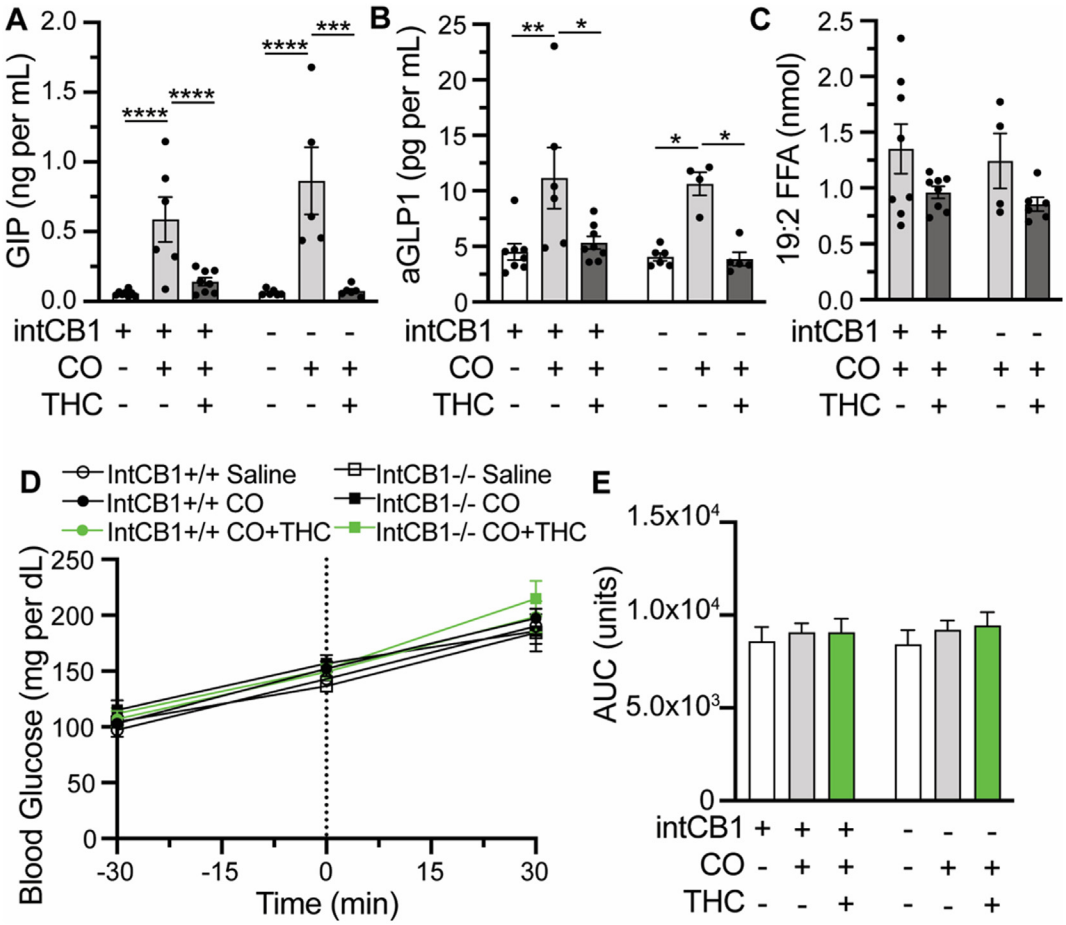

Figure 4. THC controls incretin secretion through an intestinal epithelial CB_1R-independent pathway. As previously shown, CO greatly stimulates GIP release into circulation, an effect that is blunted with THC pretreatment. Similarly, CO-induced incretin release is blocked by THC pretreatment in $intCB_1-/-$ mice [GIP: treatment main effect $F_{(2,33)} = 26.69$, $P < .0001$; aGLP1: $F_{(2,31)} = 15.23$, $P < .0001$] (A and B). As noted before, THC treatment increases gastric emptying (C, treatment main effect $F_{(1,22)} = 5.267$, $P = .0316$), resulting in more lipids reaching the intestines. Lastly, blood glucose levels throughout the experiment remained similar between conditions (D and E). Data were expressed as means ± S.E.M and analyzed by two-way ANOVA with Holm-Sidak multiple comparison test (A–C, and E) or by two-way ANOVA with repeated measures (D). n = 5–8 per condition, $^*P < .05$, $^{**}P < .01$, $^{****}P < .0001$.

the differentiation of stem cells into enteroendocrine cells – was similar between $IntCB_1-/-$ mice and $IntCB_1+/+$ mice. This result suggests the gene that regulates differentiation was unchanged between genotypes.[41] Furthermore, expression of genes for hormones *GIP*, proglucagon (pre-proprotein for GLP1), and *CCK* were similar between $IntCB_1-/-$ and $IntCB_1+/+$ control mice. In addition, no differences were found between these groups for the expression of genes for proteins that sense fatty acids in small intestinal epithelial cells (cluster of differentiation 36, *Ffar1*, *Ffar2*, *Ffar3*, *Ffar4*).

We further investigated whether a lack of CB_1Rs in the intestinal epithelium was associated with changes in the enzymatic degradation of incretins, which could confound the interpretation of results in these studies. We tested the enzymatic activity of dipeptidyl peptidase 4 (DPPIV),[37] the enzyme responsible for incretin degradation, in the plasma of $IntCB_1-/-$ and $IntCB_1+/+$ control mice. DPPIV activity was similar between treatment and genotype groups (Figure A2). Together, these results suggest that $IntCB_1-/-$ mice are likely capable of appropriately synthesizing the incretin hormones studied here, sensing dietary lipids in the intestinal lumen, and enzymatically breaking down incretin hormones in circulation.

Discussion

Incretins are hormones produced by enteroendocrine K- and L-cells that enhance insulin secretion and regulate blood sugar levels, and CB_1Rs are expressed in these cells.[16] Notably, limited studies using globally acting agonists or full-body CB_1R knockout mice suggest a role for CB_1Rs in inhibiting GIP and GLP1 secretion following a meal.[16,17,19,42] However, the functional roles of CB_1Rs located on intestinal epithelial cells in the control of incretin release and the impact that cannabinoids have in these responses require

further investigation. Here, we tested the hypothesis that CB_1Rs on intestinal epithelial cells control GIP and aGLP1 release *in vivo*. Our results indicate that *(i)* synthetic cannabinoid ligands or phytocannabinoids both inhibit fat-induced incretin secretion by a mechanism that includes activating CB_1Rs in tissues outside of the central nervous system in mice, *(ii)* synthetic cannabinoid ligands or phytocannabinoids differentially inhibit incretin secretion by a CB_1R-dependent and CB_1R-independent mechanism in the intestinal epithelium, and *(iii)* changes in the metabolism of incretins do not underlie the effects. Collectively, the results suggest that synthetic cannabinoid agonists and phytocannabinoids differentially inhibit incretin release by a mechanism that includes CB_1R-dependent and CB_1R-independent mechanisms in the intestinal epithelium and possibly other peripheral organs. Moreover, the data will be useful in the design and development of cannabinoid receptor ligands (eg, peripheral CB_1R antagonists) that control incretin release and may be beneficial for the treatment of type-2 diabetes and obesity.

Enteroendocrine cells sense luminal contents through various FFA receptors (eg, free fatty acid receptors 1–4) that are enriched in enteroendocrine populations in the intestine.[43] Thus, we utilized CO for these studies, which contain saturated and unsaturated FFAs, to stimulate incretin secretion.[44,45] Oral gavage with CO potently increased levels of GIP and aGLP1 in plasma from fasted mice, and this oil-induced incretin release was largely blocked by WIN or THC pretreatment. These results are consistent with previous research showing changes in GIP or GLP1 release following cannabinoid receptor activation in primary murine cells, mouse models, and in humans.[16,19,42] For our studies, we first utilized the peripherally-restricted neutral CB_1R antagonist, AM6545, to delineate between peripheral or central CB_1Rs in the inhibitory actions of synthetic (WIN) and phytocannabinoid (THC) cannabinoid receptor agonists. Notably, cotreatment WIN or THC with AM6545 restored the lipid-induced incretin secretion, which indicates the effects of cannabinoids are mediated through peripheral, not central, CB_1R signaling. In addition, we tested the possible effect of CB_1R activation on gastric emptying; differences in gastric emptying between the treatment conditions could affect the accessibility of the FFAs from the CO gavage to the enteroendocrine cells and alter incretin release.[46–48] THC treatment increased gastric emptying yet reduced incretin in circulation, despite more lipids entering the intestine. Thus, differences in circulating levels were not likely a result of changes in gastric emptying and lipid availability. Lastly, CB_1R signaling has been implicated in regulating blood sugar levels and insulin secretion within the endocrine pancreas, which could, in turn, affect the release of GIP or aGLP1.[2,49–52] Nonetheless, we report no changes in blood glucose levels between any conditions tested. Notably, we focused the current studies on the impact of cannabinoids on lipid-induced incretin secretion.

Next, we investigated roles for CB_1Rs in the intestinal epithelium in controlling incretin secretion using an inducible transgenic mouse model that lacks CB_1Rs in the intestinal epithelium.[15,39,40] Inducible models allow for specificity and limit the development of compensatory mechanisms due to the lack of CB_1Rs over the lifetime from birth, which occurs in whole-body CB_1R-null mice. Indeed, CB_1R signaling regulates the development of appropriate endocrine pancreas microarchitecture and overall β-cell mass by directly interfering with insulin receptor signaling.[53,54] Moreover, full-body CB_1R-null mice have altered endocrine pancreas morphology, changes in adipose tissue, and are resistant to diet-induced obesity.[52,55,56] These pathways directly interfere regulate incretin release, which makes it difficult to delineate between tissue-specific outcomes of CB_1R signaling. Under our conditions, $IntCB_1-/-$ and $IntCB_1+/-+$ mice retain similar expression of genes involved in fatty acid sensing and incretin synthesis while maintaining normal enzymatic DPPIV breakdown of circulating peptides. This result suggests that changes in the breakdown of incretins do not underlie the effects found in our studies.

Effects on GIP Secretion

Oral gavage with CO continued to induce the secretion of GIP and GLP1 into circulation in $IntCB_1-/-$ mice; however, WIN pretreatment failed to inhibit the release of GIP into circulation in $IntCB_1-/-$ mice. This result suggests that synthetic cannabinoid blockade of CO-induced GIP secretion requires CB_1Rs in the intestinal epithelium. Interestingly, THC was able to inhibit the fat-induced secretion of GIP in $IntCB_1-/-$ mice. THC broadly activates cannabinoid receptors, which include CB_2R and GPR55.[57] Thus, THC may be acting through a CB_1R-independent mechanism. Indeed, activating GPR55 with Abn-CBD has been reported to increase GIP secretion in obese mice.[58] Furthermore, endocannabinoid-like molecules such as oleoylethanolamide and 2-oleoylglycerol have been shown to increase GLP-1 secretion in mice and humans, respectively.[59,60] Thus, more research is needed to determine the specific receptor and signaling pathways that THC recruits to inhibit GIP and GLP-1 release. Nonetheless, our results suggest that this occurs by a mechanism that does not require CB_1Rs in the intestinal epithelium.

Effects on GLP1 Secretion

Interestingly, both WIN and THC pretreatment blunts GLP1 secretion following a CO gavage in $intCB_1-/-$ mice, which suggests that synthetic cannabinoid receptor agonists or phytocannabinoids affect GLP1 secretion by a mechanism that does not require CB_1Rs in the intestinal epithelium. Peripheral CB_1R control of GLP1 secretion may also involve vagal fibers and overlap with CCK-mediated gut-brain interactions. Indeed, peripheral CB_1R control of fat-induced incretin release reflects a similar pattern when compared to peripheral CB_1R regulation of CCK secretion,[14] which suggests a redundant or overlapping mechanism between

different enteroendocrine cell types. Enteroendocrine cells predominately produce a characteristic intestinal-derived hormone; however, many cells produce multiple peptides.[61–65] Open-type enteroendocrine cells (which include I and L cells) form functional synapses with afferent vagal fibers, which in turn communicate with the brain via the gut-brain axis.[66–69] Importantly, nearly half of all vagal neurons that are positive for CCK receptors also express GLP1 receptors, whereas nearly all neurons positive for GLP1 receptors contain CCK receptors mice.[70] Upon activation, GLP1 receptors on these neurons signal to the nucleus of the solitary tract to reduce food intake.[71] Notably, GIP receptors were not detected in any vagal afferent neurons indicating a unique role for GLP1 over GIP in regulating caloric intake through the vagus nerve in mice. Furthermore, CB_1R expression is colocalized to both types of CCK receptor-positive neurons (those coexpressing and lacking GLP1 receptors), as well as on other neurons not expressing CCK receptors that innervate the intestinal mucosal layer.[70] Accordingly, it is possible that CB_1Rs on these neurons – but not intestinal epithelial cells – control GLP1 secretion and may contribute to the hypophagic effects of CB_1R inhibition. A direct test to this hypothesis, however, remains to be assessed.

Peripheral CB_1R control of incretin release provides a unique therapeutic target for type-2 diabetes and obesity. Previously, global CB_1R antagonists (eg, Rimonabant) were prescribed to obese patients in Europe. Patients lost significant weight while maintaining lean body mass, and reported other beneficial effects on HDL/LDL, blood triglycerides, and decreases in hemoglobin A1c %.[72–74] CB_1Rs in the brain, however, participate in fear, anxiety, and memory.[75] Treatment with Rimonabant resulted in an increased incidence of depression and suicidal ideation, and thus was removed from the European market and was not approved by the FDA for therapeutic use in the United States.[76] Targeting peripheral CB_1Rs with nonbrain penetrant CB_1R antagonists may limit the centrally mediated side effects of central CB_1R inhibition while maintaining the beneficial therapeutic effects in reducing body mass and hyperglycemia. CB_1R regulation of fat-induced incretin release may be an important contributor to these prometabolic effects of peripherally-restricted CB_1R antagonists; however, a direct test of this hypothesis remains for future studies.

Supplementary Materials

Material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.gastha.2024.07.006.

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The animal experiments adhere to the updated ARRIVE guidelines.