Glucagon-like peptide-1 (GLP-1) secretion is classically regulated by ingested nutrients. To identify novel molecular targets controlling incretin secretion, we analyzed enteroendocrine cell pathways important for hormone biosynthesis and secretion. We demonstrate that progesterone increases GLP-1 secretion and extracellular signal–related kinase 1/2 (ERK1/2) phosphorylation in enteroendocrine GLUTag cells via mechanisms sensitive to the mitogen-activated protein kinase inhibitor U0126. The stimulatory effects of progesterone (P4) or the synthetic progestin R5020 on ERK1/2 phosphorylation were independent of the classical progesterone receptor antagonist RU486. Furthermore, a cell-impermeable BSA–progesterone conjugate rapidly increased ERK1/2 phosphorylation and GLP-1 secretion. Knockdown of the membrane progesterone receptors Paqr5 or Paqr7 in GLUTag cells eliminated the stimulatory effect of R5020 and progesterone on GLP-1 secretion. Enteral progesterone administration increased plasma levels of GLP-1, glucose-dependent insulinotropic polypeptide (GIP), and insulin, and improved oral glucose tolerance in an RU486-insensitive manner in mice: however, systemic progesterone exposure did not improve glucose homeostasis. Unexpectedly, the glucoregulatory actions of enteral progesterone did not require classical incretin receptor signaling and were preserved in Glp1r−/− and Gipr−/− mice. Intestine-restricted activation of membrane progesterone receptors may represent a novel approach for stimulation of incretin hormone secretion and control of glucose homeostasis.

**Glucagon, a proglucagon-derived peptide (PGDP) secreted from islet α-cells, is a 29-amino acid peptide hormone that plays a key role in the control of blood glucose via regulation of hepatic glucose production (1). Glucagon excess in the setting of insulin deficiency or resistance contributes to the pathophysiology of diabetes mellitus, whereas defective glucagon secretion leads to an increased risk of hypoglycemia in diabetic subjects. Conversely, glucagon-like peptide-1 (GLP-1), a 30-amino acid PGDP secreted from gut endocrine cells regulates glucose homeostasis by augmenting β-cell and inhibiting α-cell function (2). Hence, understanding the control of PGDP biosynthesis and secretion in health and disease has direct relevance for the treatment of diabetes mellitus.**

Experimental approaches for studying enteroendocrine L cells have traditionally used primary intestinal cell cultures in vitro (3,4); however, L cells often represent <1% of the cells in the culture system. Hence, immortalized enteroendocrine cell lines are commonly employed for analysis of intestinal proglucagon biosynthesis and gene transcription (4–7). More recently, targeting of reporter genes to murine enteroendocrine cells has facilitated isolation and analysis of purified populations of L cells and K cells, enabling more detailed molecular characterization of native enteroendocrine cell populations (4,8). Taken together, the use of primary cultures, cell lines, and purified endocrine cell populations examined ex vivo has greatly enhanced our knowledge of pathways regulating incretin hormone secretion.

Although islet α-cells and enteroendocrine L cells exhibit nutrient-sensitive regulation of proglucagon gene expression and PGDP secretion, the mechanisms controlling proglucagon gene transcription and PGDP secretion in these two cell types have diverged. Nutrients and insulin inhibit islet proglucagon gene expression and glucagon secretion (9) yet stimulate proglucagon gene expression and enhance PGDP secretion from gut endocrine cells in both animals and isolated cell cultures (10–13). To identify novel mechanisms regulating proglucagon biosynthesis and PGDP secretion, we carried out gene expression profiling of islet αTC-1 and enteroendocrine GLUTag cells (14,15) and identified a series of ion channels differentially expressed in GLUTag versus αTC-1 cells (15).

We demonstrate in this study that intestinal GLUTag cells express a functional progesterone receptor (PR). Progesterone increased levels of proglucagon mRNA transcripts and directly increased GLP-1 secretion from GLUTag cells in vitro. The progesterone-dependent stimulation of GLP-1 secretion was rapid, associated with extracellular signal–related kinase 1/2 (ERK1/2) activation, and insensitive to the classical progesterone antagonist RU486. Furthermore, a BSA–progesterone (P4) cell-impermeable conjugate retained the ability to activate ERK1/2 and stimulate GLP-1 secretion, and knockdown of membrane PRs in GLUTag cells eliminated the stimulatory effects of progestins on GLP-1 secretion. Furthermore, enteral but not parenteral P4 administration improved glucose tolerance and enhanced circulating levels of GLP-1 in mice. These findings identify novel intestinal targets for activating incretin hormone secretion, enabling development of ligands that target enteroendocrine membrane PRs independent of absorption and systemic exposure.

**RESEARCH DESIGN AND METHODS**

Tissue-culture medium was from Hyclone (Logan, UT), and FCS was from Invitrogen Life Technologies (Burlington, Ontario, Canada). Antibiotics, chemicals, proteinase inhibitor cocktail, phosphatase inhibitors, BSA free of fatty acids
GLP-1 secretion from GLUTag cells. GLUTag cells grown to 80% confluence in DMEM with 10% FCS were washed with PBS and preincubated for 2 h in DMEM without FCS. Media was replaced with fresh DMEM high glucose (4.5 g/l glucose/L) without FCS supplemented with vehicle (ethanol), R5020 (20 nmol/L), P4 (20 nmol/L), or BSA-P4 (20 nmol/L of P4 equivalent) for 2 h. Media and cell extracts were collected in triplicate in a microplate, and GLP-1 levels were measured using the GLP-1 radioimmunoassay (Millipore) or the total GLP-1 assay kit (Mesoscale Discovery, Gaithersburg, MD). Total GLP-1 secreted into the media was normalized to total protein content.

Animal experiments. Mouse experiments were carried out according to protocols approved by Mt. Sinai Hospital and the Toronto Centre for Phenogenomics Animal Care Committees.

Glucone tolerance tests. Age-matched C57BL/6, Glp1r−/−, and double inreceptor knockout (DIRKO) male mice (8–10 weeks old) were fasted for 4 h. On day 1 mice received a single oral dose of vehicle (100 µL of 80% PEG400, 10% Tween 80, 10% ethanol) 15 min prior to oral glucose tolerance testing (OGTT) (1.5 g/kg body weight). Four days later, the same mice received a single oral dose of P4 (100 µg in 100 µL/mouse) 15 min prior to glucose administration. Blood plasma was collected from mice fasted for 4 h at day 1, and mice were given either vehicle (100 µL of 80% PEG400, 10% Tween 80, and 10% ethanol) or the classical nuclear progesterone receptor antagonist RU486 (100 mg/kg administered in ~90–110 µL as described (21)). Sixty minutes later, mice received a single oral dose of vehicle (80% PEG400, 10% Tween 80, and 10% ethanol) (day 1) or P4 (100 µg/mouse) (day 2) followed by an oral glucose load (1.5 g/kg body weight) 15 min later. Blood glucose levels were measured by sampling from the tail vein of gently held conscious mice from 5–90 min after glucose administration. In all experiments, at the 5-min time point following glucose administration, a blood sample (150 µL) was collected and immediately mixed with 15 µL of a chilled solution containing 5000 kIU/mL Trasylol (Bayer, Toronto, Ontario, Canada), 32 mM/L EDTA, and 0.01 mM/L Diprotin A (Sigma Chemical Co.) for measurement of insulin, total GLP-1, and total GLP-1 OGT. For measurement of insulin-like growth factor-1 levels were assessed in blood samples collected 10, 15, and 20 min following oral glucose administration. For analysis of intraperitoneal progestrone action, mice were fasted for 4 h and were given a single intraperitoneal dose of either vehicle (corn oil) (day 1) or P4 (100 µg, day 2) 15 min prior to oral glucose load.

Intestinal proglucagon expression. Ten-week-old C57BL/6 male mice were given regular chow supplemented with P4 or vehicle (corn oil) for 48 h. Briefly, P4 was dissolved in corn oil, and the chow was then mixed with corn oil/P4 to obtain a diet of uniform consistency. The daily estimated dose of P4 was 10 mg/day/mouse. Mice were then killed, and jejunum and colon were cleaned with cold PBS and collected for RNA preparation. Real-time PCR was performed as described above using the assay on demand for proglucagon (Applied Biosystems). Blood was collected by cardiac puncture 15 min prior to glucose/acetaminophen administration. Plasma samples (150 L) were collected and immediately mixed with 15 µL of a chilled solution containing 5000 kIU/mL Trasylol (Bayer, Toronto, Ontario, Canada), 32 mM/L EDTA, and 0.01 mM/L Diprotin A (Sigma Chemical Co.). Plasma was obtained by centrifugation at 4°C and stored at −80°C until determination of total GLP-1 and progestrone levels.

Gastric emptying. Liquid-phase gastric emptying was assessed using the acacetaminophen absorption test (23,24). C57BL/6 and DIRKO male mice, 10–12 weeks of age, were fasted for 4 h and given a single dose of either vehicle (V; 80% PEG400, 10% Tween 80, and 10% ethanol) (day 1) or P4 (100 µg/mouse; day 2) 15 min prior to oral administration of a solution of glucose 15% and acetaminophen. Tail vein blood (50 µL) was collected into heparinized tubes at 15 and 30 min after glucose/acetaminophen administration. Plasma was separated by centrifugation at 4°C and stored at −80°C until determination of acetaminophen levels using an enzymatic-spectrophotometric assay (Diagnostic Chemicals Ltd., Oxford, CT).

Insulin tolerance test. Ten-week-old age-matched C57BL/6 and DIRKO male mice were fasted for 5 h. A single oral dose of vehicle (V; 80% PEG400, 10% Tween 80, 10% ethanol) (day 1) or P4 (100 µg/mouse) (day 2) was given 15 min prior to the administration of 1.2 U/kg of insulin (Humulin R; Eli Lilly, Toronto, Canada). Blood glucose was determined at 0, 15, 30, 60, 90, and 180 min.

Statistical analysis. Statistical significance was assessed by one-way ANOVA using Bonferroni multiple comparison post hoc test and, where appropriate, by paired Student t test using GraphPad Prism 4 (GraphPad Software, San Diego, CA). A P value <0.05 was considered to be statistically significant.
RESULTS

Differential gene expression in glucagon-producing cell lines. To identify novel pathways in enteroendocrine cells coupled to control of GLP-1 secretion, we searched for genes with functional activity potentially coupled to hormone synthesis or secretion differentially expressed in enteroendocrine cells. We identified several mRNA transcripts preferentially expressed in GLUTag versus αTC1 cells (Fig. 1A). For example, the transcription factors Cdx2, Unc4.1, and Tal1, mRNA transcripts for the ion channel Kcnq2, the glutamate receptor Grik1, the bombesin-like receptor Brs3, and the protein tyrosine phosphatase Ptpn5 were preferentially expressed in RNA from GLUTag cells. Unexpectedly, we detected robust expression of the PR in GLUTag but not in αTC1 cells (Fig. 1A).

As sex steroids have not previously been implicated in the control of GLP-1 secretion (25), we assessed whether progesterone activated proglucagon gene expression or GLP-1 secretion in GLUTag cells. To first ascertain whether the GLUTag PR mRNA transcript encodes a transcriptional competent PR, cells were transfected with the MMTV-Luc reporter plasmid and treated with the synthetic progestin R5020 or the glucocorticoid dexamethasone. R5020 and, to a lesser extent, dexamethasone significantly increased MMTV-Luc activity in GLUTag cells (Fig. 1B). In contrast, R5020 had no effect on MMTV-Luc activity in αTC1 cells; however, dexamethasone robustly induced MMTV-Luc activity in the same experiments (Fig. 1C). R5020 also increased levels of proglucagon mRNA transcripts in GLUTag cells (Fig. 1D); however, enteral P4 administration for 2 days had no effect on intestinal proglucagon mRNA transcripts in the mouse jejunum or colon (Fig. 1E). Plasma P4 levels were significantly increased, and levels of total circulating GLP-1 trended higher after chronic enteral P4 administration (Fig. 1F).

As P4 may regulate hormone secretion independent of changes in gene expression, we examined GLP-1 secretion in enteroendocrine cells. R5020 significantly stimulated GLP-1 secretion from GLUTag cells (Fig. 2A). Forskolin, a potent activator of adenylate cyclase and classical activator of GLP-1 secretion in L cells (3,6), increased both GLP-1 secretion (Fig. 2A) and produced a sustained activation of cAMP-responsive element–binding protein (CREB) phosphorylation (Fig. 2B). In contrast, R5020 rapidly but transiently increased levels of phospho-CREB at 5 min, followed by a secondary increase at 30–60 min (Fig. 2B). R5020 also increased ERK1/2 (Fig. 2C) but not AKT phosphorylation (data not shown). Furthermore, the ERK1/2 inhibitor U0126 abolished the stimulatory effect of R5020 on GLP-1 secretion (Fig. 2D). Unlike forskolin, R5020 had no effect on cyclic AMP accumulation in GLUTag cells (Fig. 2E).

FIG. 1. A functional PR is expressed in enteroendocrine cells and the gastrointestinal tract of mice. A: Examples of genes differentially expressed in enteroendocrine GLUTag cells compared with islet αTC1 cells by RT-PCR. SVT2 cells were used as a control SV40 T antigen-transformed nonendocrine cell line. PCR products were probed with an internal 32P-labeled oligonucleotide. Glutamate receptor, ionotropic, kainate 1 (Grik1), bombesin-like receptor 3 (Brs3), potassium voltage-gated channel, Shal-related family, member 2 (Kcnq2), PR, protein tyrosine phosphatase, nonreceptor type 5 (Ptpn5), cathepsin C (Ctsc), caudal type homeobox 2 (Cdx2), homeobox protein Uncx4.1 (Uncx4.1), and T-cell acute lymphocytic leukemia 1 (Tal1) were differentially expressed in GLUTag cells. GLUTag (B) and αTC1 (C) cells transfected with MMTV-Luc were treated with R5020 (20 nmol/L) or vehicle (V [ethanol]) as described in RESEARCH DESIGN AND METHODS. Luciferase activity was assessed 12 h after treatment. Dexamethasone (Dex; 10−7 mol) was used as a positive control. Results depict mean ± SD from two independent experiments each done in quadruplicate and are expressed relative to the luciferase activity measured for V-treated cells. ***P < 0.001 vs. V-treated cells. D: The progestin R5020 (20 nmol/L) increases levels of proglucagon mRNA transcripts in GLUTag cells. Proglucagon mRNA levels were assessed by qPCR following incubation of the cells with R5020 (20 nmol/L) or V for 48 h in the presence of 0.1% FCS. Data are mean ± SD of two independent experiments and are expressed relative to V-treated cells. *P < 0.01 vs. V-treated cells. E: Enteral P4 does not increase intestinal proglucagon mRNA levels in vivo. Male mice (n = 4/group) were fed a regular chow diet supplemented with P4 (daily estimated dose of P4: 10 mg/day) or V (corn oil) for 48 h. Jejunum (J) and colon (C) were collected for mRNA analysis by qPCR. Results are expressed relative to the values for J-V. F: Blood was collected from mice studied in E for the assessment of postprandial circulating P4 and total GLP-1 (tGLP-1). **P < 0.01 vs. V-treated mice.
The induction of ERK1/2 phosphorylation by R5020 and the inhibition of R5020-stimulated GLP-1 secretion by U0126 raised the possibility of a nongenomic mechanism of action. Consistent with this possibility, both R5020 and P4 rapidly increased ERK1/2 phosphorylation in GLUTag cells in the presence of the classical PR antagonist RU486 (Fig. 3A). Furthermore, we detected expression of mRNA transcripts for membrane PRs, specifically the progestin and adipok receptor family member V (Paqr5) and the progestin and adipok receptor family member VII (Paqr7) in RNA from murine jejunum and colon, as well as in GLUTag cells (Supplementary Fig. 1). To examine the importance of membrane-localized progesterone in GLUTag cells, we used BSA-P4, a P4-albumin conjugate (26). BSA-P4 rapidly increased ERK1/2 phosphorylation (Fig. 3B) and GLP-1 secretion (Fig. 3C) from GLUTag cells to levels comparable to or modestly greater than that seen with R5020 or P4. In contrast, BSA-P4, unlike R5020, had no effect on the activity of MMTV-Luc in transfected GLUTag cells (Fig. 3D).

To identify receptors required for the progestosterone-regulated control of enterocortecile GLP-1 secretion, we used siRNA to reduce expression of the PR, Paqr5, or Paqr7 in GLUTag cells (Fig. 3E). A reduction of PR mRNA by >50% unexpectedly enhanced the stimulatory effects of P4 on GLP-1 secretion (Fig. 3F). In contrast, reduction in levels of either Paqr5 or Paqr7 abrogated the ability of P4 to stimulate GLP-1 secretion (Fig. 3F). Hence, P4 stimulates GLP1 secretion via activation of both Paqr5 and Paqr7, likely in a cooperative manner in GLUTag cells.

We next examined whether P4 acutely lowers glycemia and increases plasma levels of GLP-1 in mice. A single dose of oral P4 significantly improved oral glucose tolerance, in association with increased plasma levels of insulin and GLP-1 (Fig. 4A–C). Consistent with the RU486-independent actions of progesterone on ERK1/2 phosphorylation in GLUTag cells (Fig. 3A), the P4-mediated reduction in glycemc excursion and increase in plasma levels of GLP-1 was not attenuated by concomitant RU486 administration (Fig. 4D–F). In contrast to the glucoregulatory effects of enteral P4, acute intraperitoneal P4 administration failed to improve glucose tolerance in wild-type (WT) mice (Supplementary Fig. 3) and did not increase circulating levels of plasma GLP-1 (data not shown).

As P4 stimulated GLP-1 secretion from GLUTag cells and increased plasma levels of GLP-1 following enteral administration in mice, we examined the importance of the GLP-1 receptor for the glucoregulatory actions of P4. Unexpectedly, P4 improved oral glucose tolerance and significantly increased insulin levels in both Glp1r+/+ and Glp1r+/− mice (Fig. 5A and B). Glp1r−/− mice exhibit compensatory changes in the incretin axis including upregulation of GIP secretion and enhanced GIP sensitivity (27,28); hence, we examined GIP levels following oral P4. P4 significantly increased plasma levels of GIP in WT and DIRKO mice and GIP levels also trended higher in Glp1r+/− mice (Fig. 5C). Accordingly, we assessed the importance of the GIPR and the GLP-1 receptor as targets for P4 action in Glp1r−/−:Gipr−/− (DIRKO) mice. Surprisingly, P4 significantly improved glucose tolerance in DIRKO mice.
FIG. 3. P4 stimulates ERK1/2 phosphorylation and GLP-1 secretion independent of the classical PR in GLUTag cells. A: P4-stimulated ERK1/2 phosphorylation is not inhibited by RU486 in GLUTag cells. Western blot analysis of ERK1/2 phosphorylation in cells pretreated for 15 min with the PR antagonist RU486 (1 μmol) followed by treatment for 10 or 30 min with R5020 (20 nmol), P4 (20 nmol), and epidermal growth factor (EGF: 25 ng/mL, positive control for ERK1/2 phosphorylation). Anti-HSP90 was used to monitor loading and transfer conditions. B: Covalently bound BSA-P4 increases ERK1/2 phosphorylation. GLUTag cells were treated for 10 min with the progesterin R5020 (20 nmol), P4 (20 nmol), or covalently bound BSA-P4 (20 nmol P4). Cells treated with V (ethanol) plus BSA-FA (20 nmol/L) were used as the basal control. Figure shows the densitometric analysis of ERK1/2 phosphorylation normalized to HSP90 in the same experiment and expressed relative to basal conditions. Results depict mean ± SD of two independent experiments each done in triplicate. C: BSA-P4 (20 nmol/L P4 equivalent) stimulates GLP-1 secretion in GLUTag cells. Cells were treated for 2 h with R5020 (20 nmol/L), P4 (20 nmol), or BSA-P4 (20 nmol/L of P4). Cells treated with V (ethanol) plus BSA-FA (20 nmol/L of P4) were used as the basal control. Total GLP-1 secreted in the media (pg/mL) was normalized to total cell content (μg). Results depict mean ± SD of two independent experiments, each performed in quadruplicate and expressed relative to levels of GLP-1 secreted under basal conditions. D: BSA-P4 (20 nmol/L P4) does not transactivate the MMTV-Luc promoter. GLUTag cells transfected with MMTV-Luc were treated for 12 h with R5020 (20 nmol/L), BSA-P4 (20 nmol/L of P4), V, or BSA-FA (20 nmol/L). Results are expressed relative to the luciferase activity in V-treated cells and represent the mean ± SD of a representative experiment done in triplicate. **P < 0.01 relative to vehicle-treated cells. E: siRNA knockdown reduces levels of PR, Paq5, and Paq7 mRNA transcripts in GLUTag cells. siRNA was transfected as described in RESEARCH DESIGN AND METHODS, and the efficiency of RNA knockdown was assessed by qPCR. Levels of mRNA transcripts after knockdown are expressed relative to the levels detected under basal conditions. *P < 0.05, **P < 0.01, ***P < 0.001 vs. basal conditions. F: Relative GLP-1 secretion in cells transfected with the indicated siRNAs and treated for 2 h with R5020 (20 nmol/L), P4 (20 nmol/L), or V (ethanol). Total GLP-1 secreted in the media (pg/mL) was normalized to total cell protein content (μg). Results are expressed relative to levels of GLP-1 in cells treated with V and depict mean ± SD from three independent experiments each done in triplicate. *P < 0.05, **P < 0.001 vs. basal, ###P < 0.001, small interfering PR (siPR) + P4 vs. basal + P4.

Plasma insulin levels were significantly increased after P4 administration in WT and Glp1r−/− mice 5 min after glucose challenge (Fig. 5E) and at later time points in DIRKO mice (Fig. 5F). Enteral P4 had no effect on the rate of gastric emptying in WT or DIRKO mice (Supplementary Fig. 2A) and produced a modest impairment in insulin sensitivity in both WT and DIRKO mice (Supplementary Fig. 2B and C).

**DISCUSSION**
To identify molecular mechanisms important for PGDP synthesis and secretion in gut endocrine cells, we and others have studied the genes and proteins expressed in murine GLUTag cells (6), which retain many of the properties associated with differentiated gut endocrine cells, including cAMP-dependent and nutrient-sensitive regulation of PGDP synthesis and secretion (6,29,30). The current studies demonstrate that the murine PR is expressed in cultured enteroendocrine cells and within different regions of the mouse gastrointestinal tract. Nevertheless, both P4 and membrane-restricted BSA-P4 activate ERK1/2 and GLP-1 secretion in GLUTag endocrine cells, whereas the nuclear receptor antagonist RU486 does not block the stimulatory actions of progesterone on GLP-1 secretion in mice. Moreover, enteral but not parenteral progesterone administration acutely increased GLP-1 levels and improved oral glucose tolerance in mice. Together, these findings establish a role for enteral progesterone in the pharmacological control of incretin secretion and glucose homeostasis.

Despite expression of a functional classical PR in GLUTag cells, the actions of R5020 and P4 to stimulate GLP-1 secretion were not diminished by the PR antagonist RU486 in mice. However, the MAPK inhibitor U0126 completely eliminated the stimulatory effect of progesterone on
ERK1/2 phosphorylation and GLP-1 secretion in GLUTag cells. Together, these findings strongly suggested that P4 stimulates GLP-1 secretion through nongenomic mechanisms linked to ERK1/2 signal transduction. Consistent with this possibility, we observed robust activation of both ERK1/2 phosphorylation and GLP-1 secretion in GLUTag cells using BSA-P4, a hybrid molecule that restricts passage of P4 across the cell membrane. In contrast, BSA-P4 had no effect on the transcriptional activity of MMTV-Luc in GLUTag cells, whereas R5020 robustly increased MMTV-Luc activity in the same experiments.

Limited information is available on whether and how P4 controls glucose homeostasis or islet function. A previous study demonstrated that female but not male PR2/2 mice exhibited lower fasting glucose and higher insulin levels; however, the precise mechanisms accounting for this sexual dimorphism and phenotype were not clearly elucidated (31). Our data, using both the nuclear PR antagonist RU486 in mice and BSA-P4, a hybrid molecule that restricts passage of P4 across the cell membrane. In contrast, BSA-P4 had no effect on the transcriptional activity of MMTV-Luc in GLUTag cells, whereas R5020 robustly increased MMTV-Luc activity in the same experiments.

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Acute enteral progestin administration rapidly increased plasma levels of both GIP and GLP-1. Although the majority of enteroendocrine L cells are localized to the distal gut, a substantial proportion of enteroendocrine cells within the proximal gut also exhibit the capacity for GLP-1 synthesis and secretion (35–37). Hence, the precise site of action for P4 within the gut requires further investigation.

Furthermore, experiments designed to assess the putative physiological importance of endogenous membrane PRs in the control of incretin secretion and glucose homeostasis are limited by the lack of suitable antagonists, highly specific antisera, or knockout mice.

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mechanisms in DIRKO mice treated with antisense oligonucleotides against the Gcgr (28), revealing considerable redundancy in how the gastrointestinal tract and islet β-cells maintain glucose homeostasis in response to enteral glucose administration.

Taken together, the observations that BSA-P4 stimulates GLP-1 secretion from GLUTag cells, coupled with the loss of progestin action on GLP-1 secretion following knockdown of membrane PRs strongly suggests that membrane, rather than nuclear PRs, activate GLP-1 secretion in response to P4. Furthermore, these findings are consistent with the inability of RU486, a classical nuclear PR antagonist, to diminish the GLP-1-stimulation and glucoregulatory effects observed following enteral P4 administration in mice. Our data raise interesting questions about the potential for enteral P4 or membrane PR agonists to augment incretin secretion and control glucose homeostasis under different physiological and pathophysiological situations, including type 2 diabetes. The observation that BSA-P4 enhances GLP-1 secretion in GLUTag cells, coupled with findings that enteral P4 promotes GLP-1 secretion and enhances glucose homeostasis in vivo, suggest that the enteroendocrine membrane PR system may be a potential intestinal target for selectively enhancing incretin secretion, independent of systemic progesterone exposure, for the treatment of metabolic disorders.

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G.B.F. and X.C. carried out experiments and wrote and reviewed the manuscript. M.M. analyzed microarray data and reviewed the data and manuscript. D.J.D. planned experiments, reviewed data, and wrote the manuscript. D.J.D. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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FIG. 5. P4 improves oral glucose tolerance independent of incretin receptors. Glucose excursions and area under the curve (AUC, inset) during OGTT for WT (n = 14 mice) (A), Glp1r−/− (n = 8 mice) (B), and DIRKO mice (n = 17 mice) (D). Age-matched WT, Glp1r−/−, and DIRKO mice were treated with a single oral dose of P4 (20 mmol/L; solid line) or V (vehicle) (dashed line) 15 min before an oral glucose load. For E, an independent group of DIRKO mice (n = 10 mice) was treated as described above and bled at the indicated time points following glucose administration for the assessment of plasma insulin levels during an OGTT. *P < 0.05, **P < 0.01, ***P < 0.001 vs. vehicle-treated mice.
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