GLP-1 receptor activation modulates pancreatitis-associated gene expression but does not modify the susceptibility to experimental pancreatitis in mice

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**Background** - Clinical reports link use of the GLP-1R agonists exenatide and liraglutide to pancreatitis, however whether these agents act on the exocrine pancreas is poorly understood.

**Methods** - We assessed whether the anti-diabetic agents exendin-4 or liraglutide or the DPP-4 inhibitor sitagliptin or the biguanide metformin were associated with changes in expression of genes associated with the development of experimental pancreatitis. The effects of exendin-4 when administered prior to or following the initiation of caerulein-induced experimental pancreatitis was determined. The importance of endogenous GLP-1R signaling for gene expression in the exocrine pancreas and the severity of pancreatitis was assessed in Glp1r⁻/⁻ mice.

**Results** - Acute administration of exendin-4 increased expression of egr-1 and c-fos in the exocrine pancreas. Administration of exendin-4 or liraglutide for 1 week increased pancreas weight, and induced expression of mRNA transcripts encoding the anti-inflammatory proteins PAP (RegIIIβ) and RegIIIα. Chronic exendin-4 treatment of high fat fed mice increased expression of PAP and reduced pancreatic expression of mRNA transcripts encoding for the proinflammatory proteins MCP-1, TNFα and STAT3. Sitagliptin and metformin did not significantly change pancreatic gene expression profiles. Exendin-4 administered prior to or following caerulein, did not modify the severity of experimental pancreatitis and levels of pancreatic edema and serum amylase were comparable in caerulein-treated Glp1r⁻/⁻ vs. Glp1r⁺/⁺ mice.

**Conclusions** - These findings demonstrate that GLP-1 receptor activation increases pancreatic mass and selectively modulates the expression of genes associated with pancreatitis, however activation or genetic elimination of GLP-1R signaling does not modify the severity of experimental pancreatitis in mice.
Glucagon-like peptide-1, a peptide hormone secreted by enteroendocrine cells in the distal small bowel and colon exerts a diverse set of complementary actions on islet β-cells, resulting in glucose-dependent augmentation of insulin biosynthesis and secretion (1). GLP-1 also restores glucose sensitivity to diabetic β-cells, and promotes expansion of β-cell mass via stimulation of β-cell proliferation and enhancement of β-cell survival (2). Moreover, exogenous GLP-1 administration inhibits glucagon secretion and gastric emptying and induces satiety, leading to weight loss following prolonged GLP-1R activation (3). Taken together, these actions of GLP-1 lead to significant improvement in glucose homeostasis, and have fostered the development of GLP-1R agonists, exemplified by synthetic exendin-4 (exenatide), for the treatment of type 2 diabetes (3; 4).

The majority of studies examining GLP-1 biology in the pancreas have focused on α and β-cells within the endocrine pancreas, however GLP-1 exerts a number of actions in the exocrine pancreas. GLP-1R agonists induce transdifferentiation of pancreatic exocrine cells to an endocrine cell phenotype in vitro (5) and GLP-1 inhibits hypoglycemia-induced pancreatic bicarbonate and protein secretion in the isolated perfused pig pancreas (6). Moreover, exogenous GLP-1 induces neural transmission converging on the pancreas via depolarization of neurons within the dorsal motor nucleus of the vagus that project to the exocrine pancreas (7).

Although the biology of GLP-1 action in the exocrine pancreas remains poorly understood, the clinical use of the first approved GLP-1R agonist, exenatide, has been associated with case reports of pancreatitis and some have speculated this may be related to the venomous origin of the exendin-4 peptide from the lizard (8). Although there is limited scientific information linking GLP-1 receptor activation to the pathogenesis of pancreatic inflammation, pancreatitis has also been reported in clinical trials of the human GLP-1R agonist liraglutide (9). In contrast, analysis of a health care claims database did not reveal an increased incidence of pancreatitis in hospitalized patients previously treated with exenatide vs. other anti-diabetic agents (10).

As GLP-1 inhibits pancreatic exocrine secretion (6), a putative mechanism associated with the development of pancreatitis (11), it seems possible that sustained GLP-1 receptor activation might increase the susceptibility for development of pancreatic inflammation. Moreover, exendin-4 regulates the pancreatic expression of the Reg gene family (12), and changes in expression of RegIIIβ (also known as pancreatitis-associated protein), have been associated with divergent effects on pancreatitis susceptibility and pancreatic necrosis in vivo (13; 14).

Accordingly, we examined whether GLP-1 receptor activation modulates expression of genes known to be associated with the development of inflammation or acute pancreatitis in mice. We employed two structurally distinct GLP-1R agonists, exendin-4 and liraglutide, as well as a DPP-4 inhibitor, sitagliptin, and the biguanide metformin to assess pancreatitis-associated gene expression in mice. The effect of exendin-4 administration prior to or following the administration of low dose caerulein, a chemical cholecystokinin mimic that produces secretagogue-induced pancreatitis (15) was assessed in WT mice. and changes in pancreatic gene expression and the susceptibility to pancreatitis was analyzed in Glp1r−/− mice.

**Experimental procedures—Animals:** Experiments were carried out according to protocols approved by the Animal Care
Committees of the University Health Network and Mount Sinai Hospital. Mice were housed under a 12-hour light/dark cycle with access to standard or where indicated, high fat chow (45% kcal from fat; Research Diets Inc, New Brunswick, NJ). Male C57Bl/6J mice (8-10 weeks old) were obtained from Taconic Laboratories (Hudson, NY) and were allowed to acclimatize to the animal facility for 7 days before each experiment. Glp1r<sup>−/−</sup> mice in the C57Bl/6J background were generated as described (16).

Reagents: Exendin-4 (Ex-4) was from Chi Scientific (Maynard, MA, USA), and dissolved in phosphate-buffered saline (PBS). Forskolin and caerulein were from Sigma Chemical Co. (St Louis, Missouri, USA). Caerulein was dissolved in saline (0.9% NaCl) and forskolin in dimethyl sulfoxide (DMSO). Liraglutide was from Novo Nordisk (Bagsværd, Denmark). Sitagliptin was from Merck Inc.

Induction of experimental pancreatitis: Pancreatitis was induced in 9-11 week old male C57Bl/6J mice or 3 month old male Glp1r<sup>−/−</sup> and littermate control Glp1r<sup>+/+</sup> mice using the CCK receptor agonist caerulein (17). Caerulein dose-response experiments were performed in male 9-11 week old C57Bl/6J mice to identify a submaximal dose of caerulein that would produce a low level of pancreatic inflammation. Secretagogue-induced pancreatitis was elicited by administration of 5 sequential hourly intraperitoneal (i.p.) injections of caerulein, at doses of 6, 12, 24 or 50 μg/kg body weight as described (11; 17; 18); control animals received an equal volume of saline. Mice were euthanized by CO<sub>2</sub> inhalation 1 h after the final caerulein injection. Pretreatment with exendin-4: To assess whether prior GLP-1R activation exacerbates the subsequent development of pancreatitis, male C57Bl/6J mice were injected (i.p.) twice daily with either 10 nmol/kg Ex-4 or PBS for 7 days. 16 hrs after the last exendin-4 injection, mice were administered 5 hourly i.p. injections of caerulein at 3 or 6 μg/kg body weight, as indicated, and euthanized by CO<sub>2</sub> inhalation 1 h after the final caerulein injection. Post-treatment with exendin-4: Alternatively, to assess whether a low level of pancreatitis was worsened by subsequent GLP-1R activation, male C57Bl/6J mice were administered 5 hourly i.p. injections of caerulein (6 μg/kg) followed by twice daily i.p. injections with either 10 nmol/kg Ex-4 or PBS for up to 6 days. Mice were euthanized 1, 24, 72 or 144 h (Day 0, 1, 3, and 6, respectively) after the final caerulein injection such that mice euthanized on day 1 received 2 injections of PBS or Ex-4, one in the evening (~3 h after the last caerulein injection) and one in the morning (~6 h prior to sacrifice). Similarly, the final injection with Ex-4 or PBS was 6 h prior to sacrifice for all remaining mice (Day 3 and 6). To determine whether loss of GLP-1R signaling protected mice from development of experimental pancreatic inflammation, pancreatitis was induced in 3 month old male Glp1<sup>+/+</sup> and Glp1r<sup>−/−</sup> mice by administration of 5 hourly i.p. injections of caerulein (6 μg/kg). Mice were euthanized by CO<sub>2</sub> inhalation 1 h after the final caerulein injection.

Acute peptide administration and high fat (HF) feeding studies: To assess whether GLP-1 receptor activation regulates gene expression in the exocrine pancreas of normal mice, male C57Bl/6J mice were injected subcutaneously (s.c.) with a single dose of Ex-4 (1 μg), Ex-4 + 15% glucose (G+X), or PBS and mice were euthanized 30, 45, 60 or 90 min later by CO<sub>2</sub> inhalation. To determine whether HF feeding and induction of insulin resistance (19) modifies the effects of exendin-4 on the exocrine pancreas, 4-week-old wild-type male mice were fed either standard rodent chow or HF diet (45% kcal from fat) for 8 weeks; during the last 4 weeks mice on HF diet were given twice daily i.p.
injections of PBS or Ex-4 (24 nmol/kg). Separately, to assess the impact of diabetes on the GLP-1R-dependent regulation of exocrine pancreatic gene expression, 13 week-old male C57BL/6 mice were maintained on a HF diet (HFD) for 8 weeks, given one dose of streptozotocin (100 mg/kg), and after 4 more weeks were allocated to 4 treatment groups for an additional 8 weeks of: 1) HFD and twice daily i.p. injections of PBS, 2) HFD and Metformin (500 mg/kg/day) provided in the drinking water, 3) HFD and twice daily i.p. injections of Ex-4 (3 nmol/kg), or 4) HFD and sitagliptin (~ 370 mg/kg/day) provided in the diet.

**RNA isolation and quantitative real-time RT-PCR:** RNA from pancreatic tissue was extracted using TRIzol reagent; after first-strand cDNA synthesis real-time quantitative PCR was carried out as described (22). Quantification of transcript levels was performed with the ABI PRISM SDS 2.1 software. 18S rRNA or cyclophilin mRNA was used for normalization as expression of both remained unaltered regardless of treatment.

**Histology:** Paraffin-embedded tissues were sectioned (5 μm) and stained with haematoxylin & eosin. Immunohistochemistry was carried out using indirect immunoperoxidase detection with NovaRED substrate (Vector Laboratories, Burlington, Ontario, Canada) followed by hematoxylin counterstaining. Primary rabbit polyclonal antibodies were used at dilutions recommended by the manufacturer and included: rabbit anti-c-fos (Sigma-Aldrich), rabbit anti-egr-1 (Santa Cruz Biotechnology, Santa Cruz, CA), and rat anti-mouse neutrophils (MCA771GA, AbD Serotec, Kidlington, Oxford, UK).

**Ex-vivo pancreas preparation and cAMP assay:** Pancreatic fragments were prepared from C57Bl/J6 mice. Briefly the pancreas was rapidly cut into small pieces, cultured in DMEM + 1% BSA + 10% trasylol, washed 3x with DMEM + 1% BSA + 5% trasylol, resuspended in DMEM + 1% BSA + 10% trasylol and aliquoted such that each tube contained ~ 4% of the entire pancreas. Preparations were then stimulated with 100 nM Ex-4 or 20 μM forskolin for 15 or 30 min at 37 °C and frozen on dry ice. Samples were thawed, sonicated with ice-cold ethanol (65% final concentration), and cellular debris removed by centrifugation at 13,000 rpm. cAMP was measured from dried aliquots of ethanol extracts using a cAMP radioimmunoassay kit (Biomedical Technologies, Stoughton, MA).
Statistical analysis: Statistical significance was assessed by one-way or two-way ANOVA using Bonferroni’s multiple comparison posthoc test and, where appropriate, by unpaired Student’s t test using GraphPad Prism 4 (GraphPad Software, San Diego, CA). A P value of <0.05 was considered to be statistically significant.

RESULTS

Ex-4 acutely increases egr-1 and c-fos expression in the exocrine pancreas: Mice treated with GLP-1R agonists exhibit a significant increase in pancreas weight (23) that cannot be attributed solely to increased β-cell mass. To determine whether GLP-1R activation induces a program of gene expression in the exocrine pancreas associated with development of pancreatic growth or inflammation, we examined whether Ex-4 enhanced the expression of immediate early genes (IEGs) known to play important roles in regulating cell proliferation. Ex-4 rapidly and robustly increased pancreatic levels of egr-1 and c-fos (Figure 1A), c-myc and junB mRNA transcripts (Supplementary Figure 1 available in the online only appendix at http://diabetes.diabetesjournals.org).

Moreover, the egr-1 protein was not upregulated in islets, but was localized exclusively to nuclei of cells within the exocrine pancreas (Figure 1B), whereas nuclear c-fos expression was observed in both islets and exocrine tissue (Figure 1C). Hence GLP-1R activation induces a robust induction of gene and protein expression in the exocrine pancreas.

Treatment with Ex-4 induces expression of PAP: Egr-1 is an early response gene that encodes a transcription factor which regulates cell proliferation, growth, and apoptosis (24). Induction of Egr-1 expression occurs early in the course of caerulein-induced pancreatitis and inflammation-related gene expression is attenuated in egr-1−/− mice with experimental pancreatitis (25). Accordingly, we examined whether agents that activate GLP-1Rs upregulate expression of genes associated with pancreatitis. Mice were treated with Ex-4 for 1 week (Supplementary Figure 2 in the online appendix) and the expression of pancreatitis-associated genes was assessed by quantitative RT-PCR. Ex-4 (1 or 10 nmol/kg twice daily for 1 week) increased pancreas weight (Fig 2A) but had no effect on levels of mRNA transcripts encoding the pro-inflammatory mediators mcp-1, ICAM-1, STAT3, ndrg1 or ifitm3 or transcription factors (egr-1, ATF-3, mist1, c-fos, c-myc and junB) associated with acute pancreatitis (Fig 2B and Supplementary Table 1 in the online appendix). In contrast, Ex-4 markedly increased pancreatic expression of the anti-inflammatory gene PAP (Reg IIIβ) and Reg IIIα (Fig 2B). The increase in pancreatic weight and induction of PAP and RegIIIα gene expression was not specific to Ex-4, but was also observed in mice treated with the human GLP-1R agonist liraglutide (Figure 2C and D). These actions required a functional GLP-1R as liraglutide had no effect on pancreatic weight or induction of gene expression in Glp1r−/− mice (Fig 2C,D). Moreover, liraglutide reduced the expression of the pro-inflammatory transcription factor, STAT3, in WT mice, but not in Glp1r−/− mice (Fig 2D). The expression levels of most pancreatitis-associated mRNA transcripts were comparable in Glp1r−/− vs Glp1r+/− pancreas, however basal levels of SOCS3 and STAT3 mRNA transcripts were lower in Glp1r−/− mice (Fig 2D). Taken together, these findings demonstrate that structurally distinct GLP-1R agonists produce changes in pancreatic mass and gene expression, actions requiring a functional GLP-1 receptor.

Effect of Ex-4 pretreatment on susceptibility to caerulein-induced pancreatitis: To assess whether antecedent activation of the GLP-1R increases the severity of pancreatitis, mice were treated
twice daily with Ex-4 for 1 week prior to exposure to caerulein (Figure 3). A low dose of caerulein was employed for these studies (3 or 6 ug/kg) based on preliminary experiments designed to induce a detectable yet submaximal inflammatory response in the pancreas (Supplementary Figure 3 in the online appendix). Ex-4 significantly increased pancreas weight (Figure 3B) whereas caerulein alone produced a small increase in pancreas weight (Fig 3B) and a modest increase in tissue edema (Fig 3C). Caerulein consistently produce a low but detectable level of inflammation, as evidenced by increased pancreatic edema, elevated serum amylase, neutrophil infiltration, and upregulated Egr-1 and c-Fos expression in the exocrine pancreas (Supplementary Figure 3-4 in the online appendix, and data not shown). Intriguingly, serum amylase levels were lower in mice pretreated with Ex-4 prior to exposure to 3 μg/kg caerulein (Fig 3D). Ex-4 did not significantly modulate levels of most pancreatitis-associated mRNA transcripts regulated by caerulein administration with the exception of Reg IIIα and SOCS3 which were further induced in Ex-4-treated mice exposed to caerulein (Fig 3E). Consistent with previous findings (Fig 2A), treatment with Ex-4 for 1 week significantly increased the transcript levels of the anti-inflammatory protein PAP (Fig 3E), in the presence or absence of caerulein.

Loss of GLP-1R signaling does not modify the severity of caerulein-induced pancreatitis: To determine whether endogenous GLP-1R signaling influences susceptibility to caerulein-induced pancreatitis, we assessed the effects of a submaximal dose of caerulein on gene expression and severity of pancreatitis in Glp1r−/− vs. Glp1+/− mice. Pancreas weight, edema, and serum amylase were not significantly different in caerulein-treated Glp1r−/− vs. Glp1+/− mice (Fig 4B-D). Furthermore, GLP-1R genotype had no effect on the expression of pancreatic genes known to be induced by caerulein, including SOCS3, mcp-1, egr-1, ATF-3 and c-fos (Fig 4E). Intriguingly, caerulein-induced levels of PAP, RegIIIα, c-myc and junB mRNA transcripts were significantly greater in Glp1r−/− mice (Fig 4 E).

Ex-4 does not affect recovery from or severity of caerulein-induced pancreatitis: To determine whether GLP-1R agonists exacerbate the severity or prolong the recovery following initiation of acute pancreatitis, mice were treated with caerulein followed by 10 nmol/kg of Ex-4 twice daily for up to 6 days (Figure 5A). Mice were euthanized for analysis at 1 h, 24 h, 72 h and 6 days after the final injection of caerulein. Caerulein transiently but significantly increased serum amylase (Fig 5B, Day 0), pancreatic edema (Fig 5C, Day 0) and relative pancreas weight (Fig 5D, day 1). However, serum amylase and pancreatic edema returned to basal levels after 24 hrs, independent of Ex-4 treatment (Fig 5B and C). Similarly, levels of mRNA transcripts encoding the pro-inflammatory proteins MCP-1, IL-6, ndrg1 and ifitm3 were rapidly induced by caerulein, but returned to basal levels within 24 hrs independent of Ex-4 treatment (Fig 5E). Levels of STAT3 and ifitm3 transcripts were elevated to a greater extent in Ex-4 treated mice (Fig 5E) and mRNA transcripts for the anti-inflammatory proteins PAP and SOCS3 remained elevated in Ex-4 treated mice. In contrast, mRNA transcripts for c-fos, c-myc, and junB were transiently increased in the pancreas of caerulein-treated mice, but were not affected by concomitant Ex-4 treatment (Fig 5E) whereas levels of pancreatic mRNA transcripts for mist1 and egr1 were modestly but significantly higher in Ex-4 treated mice.

Exendin-4 modulates pancreatic gene expression in mice with metabolic stress: To determine whether GLP-1R activation modified the expression of pancreatitis-associated genes in the setting of
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A mild metabolic stress associated with the development of insulin resistance (19), mice were placed on a high fat diet (HFD) and treated twice daily with saline or Ex-4. (Fig 6A). Consistent with previous observations, Ex-4 prevented weight gain (Fig 6C) and significantly increased pancreas weight (Fig 6B), but did not affect levels of serum amylase (Fig 6D). Moreover HFD mice treated with saline, but not Ex-4, exhibited a significant increase in the transcript levels of pancreatitis-associated genes including mcp-1, STAT3, egr-1, and ATF-3 as well as the exocrine-specific transcription factor mist1 (Fig 6E). In contrast, Ex-4, (but not saline) administration significantly increased levels of mRNA transcripts for PAP, c-fos, c-myc and junB (Fig 6E).

We next determined whether metformin, sitagliptin, or Ex-4, anti-diabetic agents associated with enhanced GLP-1 receptor activation through different mechanisms (3; 4; 26), modulated pancreatitis-associated gene expression profiles in HF-fed diabetic mice. Ex-4, and to a lesser extent the DPP-4 inhibitor sitagliptin, but not metformin increased PAP mRNA transcript levels in diabetic mice (Figure 7). Neither Ex-4, metformin or sitagliptin treatment modulated the expression of other genes associated with pancreatitis including SOCS3, egr-1, STAT3, c-myc or junB (Fig 7B). Finally, as exendin-4 was reported to exhibit high affinity binding to guinea pig pancreatic acinar membranes and stimulate cyclic AMP formation through a distinct receptor that preferentially recognized exendin-4 relative to GLP-1 (27), we assessed cyclic AMP formation in slices from WT mouse pancreas. Forskolin, but not Ex-4 rapidly stimulated cyclic AMP accumulation in pancreatic slices ex vivo (Figure 8).

DISCUSSION

There is currently limited data regarding putative effects of glucagon-like peptides on the function of the normal or inflamed exocrine pancreas. Intriguingly, co-administration of glucagon and careulein attenuated the increase in pancreatic weight and amylase expression seen in rats treated with careulein alone (28). Similarly, oxyntomodulin, a peptide structurally related to both glucagon and GLP-1, was shown to be 10-fold more potent than glucagon in the suppression of rat pancreatic exocrine secretion in the basal state, or following administration of caerulein (29). Our results extend these findings by demonstrating that although acute and chronic GLP-1 receptor activation modulates a gene expression program in the exocrine murine pancreas, GLP-1 receptor activation does not predispose to or exacerbate experimental pancreatitis in mice.

Several lines of evidence imply that exocrine cells express a functional GLP-1 and/or exendin receptor. Guinea pig pancreatic acini contain high affinity binding sites for exendin-4, and exendin-4 binding was displaced from acinar cells by co-incubation with native GLP-1 (27). Furthermore, both exendin-4 and GLP-1 stimulated cAMP formation but not amylase release in dispersed guinea pig pancreatic acini (27; 30) and these stimulatory actions on cAMP were blocked by the GLP-1R antagonist exendin(9-39) (30; 31). Similarly, exendin-4 stimulated cyclic AMP formation in rat pancreatic slices, and potentiated calcium ionophore-, neurotransmitter- or CCK-induced amylase release in vitro (32). In contrast, we found that forskolin, but not exendin-4, rapidly increased cAMP formation in murine pancreatic fragments. Whether these findings reflect species-specific differences in the expression or signaling of functional pancreatic exendin-4/GLP-1 receptors requires further investigation.

We employed the CCK agonist caerulein for studies of experimental pancreatitis as previous data demonstrated a
functional interaction of CCK and GLP-1 signaling pathways in the endocrine and exocrine pancreas (32; 33). Although the expression of most pancreatitis-associated genes was not further modified by administration of GLP-1R agonists, we consistently detected up-regulation of PAP following chronic administration of exendin-4 to mice, in the presence or absence of caerulein administration. Similarly, liraglutide also induced PAP expression, in a GLP-1 receptor-dependent manner, and basal levels of pancreatic PAP mRNA transcripts were modestly reduced in Glp1r−/− mice.

PAP has been shown to be mitogenic, anti-apoptotic and anti-inflammatory and is strongly induced early in the course of inflammatory diseases such as pancreatitis, Crohn’s disease and ulcerative colitis (14). Moreover, inhibition of PAP gene expression in rats augments the severity of acute pancreatitis (34) and PAP knockout mice exhibit more extensive inflammation following induction of caerulein-induced pancreatitis (13), suggesting a protective role for PAP in the inflammatory response to cellular injury. Hence, the induction of PAP following GLP-1 receptor activation may represent a compensatory mechanism that serves to limit damage to the exocrine pancreas.

Our studies of GLP-1R activation on gene expression and the development of and/or recovery from pancreatitis were motivated in part, by clinical reports of acute pancreatitis in diabetic patients treated with GLP-1R agonists (8; 10). Our findings do not support the hypothesis that GLP-1R activation sensitizes the murine pancreas to the development of pancreatic inflammation. Similarly, we did not detect any difference in the extent of pancreatic inflammation in Glp1r−/− mice. Taken together, the available data demonstrate that GLP-1R activation leads to increases in mass and changes in expression of pancreatitis-associated genes, but does not modify pancreatitis susceptibility or severity in the murine pancreas. Whether GLP-1R activation modifies gene expression, enzyme secretion, or pancreatic inflammation in the human pancreas requires further analysis.

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Figure 1: Acute induction of egr-1 and c-fos transcripts by Ex-4. Male C57Bl/6J mice were s.c. injected with a single dose of Ex-4 (1 μg) or vehicle alone (PBS). A) Total RNA was isolated from pancreas 45 minutes after injections, reverse transcribed and the levels of the indicated transcripts were determined by real-time PCR, normalized to 18S rRNA content and shown relative to the control (PBS) treated group. Results are expressed as means ± S.E. *** p<0.001, ** p<0.01 PBS vs. Ex-4 treated mice. n=4 in each group. Immunohistochemical localization of Egr-1 (B) and c-Fos (C) in the pancreas of mice treated for 45 min with vehicle (PBS) or Ex-4. Photomicrographs are representative of 4 mice per group. Islets are represented with an i. Magnification x200 or x400.

Figure 2: GLP-1R agonists increase pancreatic weight and regulate gene expression in a GLP-1R-dependent manner. A) Pancreas weight of mice treated twice daily with the indicated doses of Ex-4 for 1 week is shown as a percentage of the final body weight (left panel) or absolute pancreas weight (right panel). Results are expressed as means ± S.E. * p<0.05 PBS vs Ex-4 treated mice. n=5 in each group. B) Expression profiles of genes associated with pancreatitis in the pancreas of mice treated twice daily for 1 week with the indicated doses of Ex-4 (0.1-10 nmol/kg). Total RNA was isolated from pancreas samples, reverse transcribed and the levels of the indicated transcripts were determined by real-time quantitative PCR, normalized to levels of 18S, or cyclophilin mRNA transcripts (for SOCS3, STAT3, junB, ndrg1 and ifitm3) and shown relative to the control (PBS) treated group. Results are expressed as means ± S.E. *** p<0.001, ** p<0.01 PBS vs. Ex-4 treated mice. n=5 in each group. Pancreas weight shown as a percentage of the final body weight (left panel), or absolute pancreas weight (right panel) (C), and expression profiles of genes associated with pancreatitis (D) in the pancreas of WT and Glp1r−/− mice treated twice daily for 1 week with 75 μg/kg liraglutide. Levels of mRNA transcripts were determined by real-time quantitative PCR, normalized to the 18S rRNA content and shown relative to control (PBS) treated WT mice. Results are expressed as means ± S.E. ** p<0.01, * p<0.05, PBS vs. liraglutide treated mice, ## p<0.01, # p<0.05, WT vs. Glp1r−/− mice. n=3 in each group.

Figure 3: Effect of prior exposure to Ex-4 on the severity of caerulein-induced acute pancreatitis. Mice were treated twice daily with PBS or 10 nmol/kg Ex-4 for one week followed by 5 hourly injections with the indicated concentrations of caerulein. A) Schematic representation of experimental design. Pancreas weight (B) shown as a percentage of the final body weight (left panel), or absolute pancreas weight (right panel), pancreatic water content (edema) (C), and serum amylase (D) were assessed in mice 6 h after the initial caerulein injection. Shown are means ± S.E., or for (D): means ± S.E. left panel, and individual serum amylase levels right panel. ## p<0.01, # p<0.05 PBS vs. Ex-4 treatment, * p<0.05 saline vs. caerulein treatment. n=4 in each group. E) Expression profiles of pancreatitis-associated genes in the pancreas of mice treated with PBS or 10 nmol/kg Ex-4 for 1 week prior to caerulein administration as indicated above. Levels of the indicated transcripts are plotted relative to levels from pancreata of control (PBS 1 week + saline treatment) group as determined by real-time quantitative PCR normalized to the 18S rRNA content, or cyclophilin mRNA levels (for STAT3, SOCS3, ndrg1 and ifitm3). Results are expressed as means ± S.E. # p<0.05 PBS vs. Ex-4 treatment, *** p<0.001, ** p<0.01, * p<0.05 saline vs. caerulein treatment. n=4 mice in each group.
Figure 4: Susceptibility of Glp1r+/− mice to caerulein-induced pancreatitis. Glp1r+/− (WT) and Glp1r−/− mice were administered 5 hourly injections of 6 μg/kg caerulein. A) Schematic representation of experimental design. Pancreas weight (B) shown as a percentage of the final body weight (left panel), or absolute pancreas weight (right panel), pancreatic water content (edema) (C) and serum amylase (D) were assessed in mice 6 h after the initial caerulein injection. Shown are means ± S.E., or for (D): means ± S.E. left panel, and individual serum amylase levels right panel. *** p<0.001 saline vs. caerulein treatment. n=5 (saline) or n=4 (caerulein) mice for each genotype. E) Expression profiles of pancreatitis-associated genes in the pancreas of WT and Glp1r−/− mice following caerulein administration. Levels of mRNA transcripts relative to the WT control (saline) treated group are shown normalized to 18S rRNA content. Results are expressed as means ± S.E. *** p<0.001, ** p<0.01, * p<0.05 saline vs. caerulein treatment. ## p<0.01 Glp1r+/+ vs. Glp1r−/− mice. n=5 (saline) or n=4 (caerulein) mice for each genotype.

Figure 5: Ex-4 does not modify severity of or recovery from, caerulein-induced pancreatitis. Mice were administered 5 hourly injections with 6 μg/kg caerulein followed by twice daily injections with PBS or 10 nmol/kg Ex-4. A) Schematic representation of experimental design. Serum amylase (B), pancreatic water content (edema) (C) and pancreas weight (D), shown as a percentage of the final body weight (left panel), or absolute pancreas weight (right panel), were assessed in mice 6 h after the initial caerulein injection (Day 0), or 24 h (Day 1), 72 h (Day 3), or 144 h (Day 6) after the final caerulein injection. Day 1 mice received two treatments with either PBS or Ex-4. Shown are means ± S.E. ## p<0.01, # p<0.05 PBS vs. Ex-4 treatment. *** p<0.001, ** p<0.01, * p<0.05 saline vs. caerulein treatment. n=4 mice in each treatment group.

Figure 6: Exendin-4 modulates pancreatic gene expression in high fat fed mice. A) A) Schematic representation of experimental design. Pancreas weight (B), body weight (C), serum amylase (D), and pancreas gene expression profiles (E) of mice treated twice daily with PBS or 24 nmol/kg Ex-4 for 1 month on normal chow (NC) or high fat (HF) diet as described (23). Results are expressed as means ± S.E. or for (D): means ± S.E. left panel, and individual serum amylase levels right panel. E) Shown are the levels of the indicated transcripts relative to control (PBS treated mice on normal chow) as determined by real-time quantitative PCR normalized to cyclophillin mRNA levels (for STAT3, SOCS3, ndrg1 and ifitm3). Results are expressed as means ± S.E. ### p<0.001, ## p<0.01, # p<0.05 PBS vs. Ex-4 treatment, *** p<0.001, ** p<0.01, * p<0.05 saline (Day 0) vs. caerulein treatment. n=4 in each treatment group.

Figure 7: Exendin-4 increases PAP gene expression in diabetic mice. Mice on a high fat diet for 8 weeks were treated with a single dose of streptozotocin (100 mg/kg). After 4 weeks of hyperglycemia on a high fat diet, mice were then treated with metformin (500 mg/kg/day), Ex-4 (3 nmol/kg), or sitagliptin (~370 mg/kg/day) for an additional 8 weeks on a
high fat diet. Mean glucose levels at end of study ranged from 10 to 14 to 16 mm for exendin-4 vs. metformin vs. sitagliptin-treated mice. A) Schematic representation of experimental design. B) Expression profiles of pancreatitis-associated genes in the pancreas of mice treated as indicated above. Shown are the levels of mRNA transcripts relative to control (PBS treated mice on normal chow) as determined by real-time quantitative PCR normalized to cyclophilin mRNA levels. Results are expressed as means ± S.E. * p<0.05 PBS vs. Ex-4 treated mice on a HF diet + streptozotocin treatment. n=4-6 in each group.

**Figure 8: Ex-4 does not increase cAMP levels in pancreatic fragments ex vivo.** The entire pancreas was rapidly digested and treated with 100 nM Ex-4 or 20 μM forskolin (Fsk) for 15 or 30 min. Cell extracts were analyzed for cAMP content. cAMP levels were normalized to total protein in pancreatic extracts. Results are expressed as means ± S.E. *** p<0.001 PBS vs. Fsk treated pancreas preparations. n=3 in each group.
GLP-1 and experimental murine pancreatitis

Figure 2

A) Pancreas weight (% BW) and absolute pancreas weight

B) Anti-inflammatory:
- PAP (Reg IIIβ)
- Reg IIIα
- SOCS3

Pro-inflammatory:
- MCP-1
- ICAM-1
- STAT3
- ndrg1
- ifitm3

Transcription factors:
- egr-1
- ATF3
- mist1 (Bh1hb8)
- c-fos
- c-myc
- junB
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C) Pancreas weight (% BW) and absolute pancreas weight

D) Anti-inflammatory:
- PAP
- RegIIIα
- SOCS3

Transcription factors:
- egr-1
- ATF3
- STAT3
- c-fos
- c-myc
- junB
Figure 3

A) 

B) pancreas weight (% BW)

C) absolute pancreas weight

D) Edema

D) serum amylase

serum amylase
E) Anti-inflammatory:

- **PAP**
- **Reg IIIα**
- **SOCS3**

Pro-inflammatory:

- **MCP-1**
- **IL-6**
- **STAT3**
- **nrdg1**
- **ifitm3**
- **TNFα**

Transcription factors:

- **egr-1**
- **ATF3**
- **mist1**
- **c-fos**
- **c-myc**
- **junB**
Figure 4

A) 

B) 

pancreas weight (% BW)  absolute pancreas weight

C) 

Edema

D) 

serum amylase  serum amylase

(normalized for total protein) (normalized for total protein)
GLP-1 and experimental murine pancreatitis

Figure 5

A) Day 0

B) serum amylase

C) Edema

D) pancreas weight (% BW)

absolute pancreas weight
GLP-1 and experimental murine pancreatitis

E) Anti-inflammatory:

- **PAP**
- **SOCS3**

Pro-inflammatory:

- **MCP-1**
- **IL-6**
- **STAT3**
- **ntrg1**
- **ifitm3**

Transcription factors:

- **egr-1**
- **mist1**
- **c-fos**
- **c-myc**
- **junB**
Figure 6

A) Time course of experimental pancreatitis with high-fat diet (HFD) and saccharin (sac) treatment. The timeline shows weekly intervals from week 0 to week 12, with age (weeks) on the x-axis and 0 weeks leading to 12 weeks.

B) Absolute pancreas weight comparison among groups: PBS NC, PBS HF, and Ex-4 HF.

C) Body weight comparison among groups: PBS NC, PBS HF, and Ex-4 HF.

D) Serum amylase levels normalized for total protein across groups: PBS NC, PBS HF, and Ex-4 HF.
Figure 7

A)

GLP-1 and experimental murine pancreatitis

B)

Anti-inflammatory:

- PAP
- SOCS3

Transcription factors:

- egr-1
- STAT3
- c-myc
- junB
Figure 8

The graph shows the cAMP (pmoles) normalized for total protein after treatment with different conditions: PBS, Ex-4, and Fsk. The treatment times are 15 min and 30 min. The bars indicate statistical significance with *** indicating a p-value < 0.001.