Glucagon-like-peptide-1 receptor is present in pancreatic acinar cells and regulates amylase secretion through cAMP

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Hou Y, Ernst SA, Heidenreich K, Williams JA. Glucagon-like peptide-1 receptor is present in pancreatic acinar cells and regulates amylase secretion through cAMP. Am J Physiol Gastrointest Liver Physiol 310: G26–G33, 2016. First published November 5, 2015; doi:10.1152/ajpgi.00293.2015.—Glucagon-like peptide-1 (GLP-1) is a glucocincretin hormone that can act through its receptor (GLP-1R) on pancreatic β-cells and increase insulin secretion and production. GLP-1R agonists are used clinically to treat type 2 diabetes. GLP-1 may also regulate the exocrine pancreas at multiple levels, including inhibition through the central nervous system, stimulation indirectly through insulin, and stimulation directly on acinar cells. However, it has been unclear whether GLP-1R is present in pancreatic acini and what physiological functions these receptors regulate. In the current study we utilized GLP-1R knockout (KO) mice to study the role of GLP-1R in acinar cells. RNA expression of GLP-1R was detected in acutely isolated pancreatic acini. Acinar cell morphology and expression of digestive enzymes were not affected by loss of GLP-1R. GLP-1 induced amylase secretion in wild-type (WT) acini. In GLP-1R KO mice, this effect was abolished, whereas vasoactive intestinal peptide-induced amylase release in KO acini showed a pattern similar to that in WT acini. GLP-1 stimulated cAMP production and increased protein kinase A-mediated protein phosphorylation in WT acini, and these effects were absent in KO acini. These data show that GLP-1R is present in pancreatic acinar cells and that GLP-1 can regulate secretion through its receptor and cAMP signaling pathway.

**GLUCAGON-LIKE PEPTIDE-1 (GLP-1)** is a potent glucocincretin hormone secreted by intestinal L cells in response to nutrient ingestion (9). GLP-1 has been shown to increase insulin secretion (16, 22, 25), insulin gene expression (10), and proliferation of pancreatic β-cells (4, 5, 41). The incretin effect on insulin secretion is dependent on plasma glucose. It has also been reported that GLP-1 and its mimetics exhibit cardioprotective effects (3, 29), inhibit gastrointestinal secretions and motility (27, 49), and regulate food intake and appetite (14, 43). GLP-1 exerts its actions by binding to its specific receptor (GLP-1R) on target cells (7, 12). It has been well characterized that GLP-1 or GLP-1 mimetics, such as exendin-4, bind to GLP-1R and lead to G-protein-mediated elevations of cAMP and activation of protein kinase A (PKA) (13, 34, 51). The GLP-1R signaling pathway has been shown to be involved in other nonglycemic actions, such as regulation of inflammation (15), lipid metabolism (18), and blood pressure (19).

While GLP-1-based therapies for type 2 diabetes have been adopted in clinical practice, pancreatitis has been reported as a rare side effect of exenatide and liraglutide (GLP-1 mimetics) therapy (1, 8, 11, 28). However, no convincing evidence of a mechanism by which GLP-1-based therapies induce pancreatitis has emerged. A limiting factor is the lack of knowledge as to whether GLP-1R is expressed in pancreatic acinar cells, which remains controversial (20, 36, 45, 48). Earlier studies indicate that iodinated GLP-1 and its mimetic exendin-4 can bind to specific receptors on guinea pig pancreatic acinar cells; GLP-1 potentiated the effect of cholecystokinin (CCK) to induce amylase release but, by itself, did not stimulate amylase secretion (35, 40). Recently, expression of GLP-1R in human pancreatic acinar cells was shown by immunohistochemistry using a novel optimized antibody; GLP-1R was shown to be bound to the plasma membrane of the acinar cells (48). Furthermore, exendin-4 was shown to increase pancreatic mass through GLP-1R in the exocrine pancreas, although this was believed not to be a direct effect (20). In the current study we utilized GLP-1R knockout (KO) mice to investigate the presence and physiological function of GLP-1R in pancreatic acinar cells. We found that GLP-1R mRNA is expressed in isolated mouse acini. GLP-1 induces amylase secretion in isolated wild-type (WT) acini but has no effect in GLP-1R KO acini; other secretagogues induce similar amylase release in WT and KO acini, suggesting the specificity of GLP-1’s effect on GLP-1R in stimulating secretion. GLP-1-induced intracellular signaling was also impaired in GLP-1R KO acini. Our study provides strong evidence that GLP-1R is present in pancreatic acini and can mediate secretion of digestive enzymes.

**MATERIALS AND METHODS**

**Ethics statement.** All experimental protocols were approved by the University of Michigan Committee on the Use and Care of Animals. Carbon dioxide inhalation was used for euthanasia.

**Mice and reagents.** GLP1r”–/– C57BL/6 mice were provided by Dr. Daniel J. Drucker (University of Toronto) (39). Collagenase NB8 broad range from *Clostridium histolyticum* was purchased from SERVA (Heidelberg, Germany); GLP-1-(7–36), vasoactive intestinal peptide (VIP), and soybean trypsin inhibitor from Sigma; 8-(4-chlorophenylthio)-2′-O-Me-cAMP (pCPT-cAMP) from BioLog (Bremen, Germany); rabbit polyclonal anti-elastase and anti-ribonuclease antibodies from Rockland Immunochemicals (Gilbertsville, PA); rabbit polyclonal anti-chymotrypsin and mouse monoclonal anti-lipase antibodies from Santa Cruz Biotechnology (Dallas, TX); rabbit polyclonal anti-amylose antibody from Sigma; rabbit polyclonal anti-phosphorylated (Ser/Thr) PKA substrate antibody from Cell Signaling Technology; Oregon Green 488-phalloidin from Life Technologies; O Me-cAMP from BioLog (Bremen, Germany); and anti-glucagon-like peptide-1 receptor antibodies from Santa Cruz Biotechnology (Dallas, TX).
Isolation of pancreatic acini and islets. Pancreatic acini from 6- to 8-wk-old male GLP-1R KO or WT C57BL/6 mice were isolated by enzymatic digestion with collagenase followed by mechanical shearing as previously described (52). After filtration through a 200-μm Nitex mesh, the mixture of acini and islets was purified by sedimentation in centrifugation buffer containing 4% BSA. Pancreatic islets and acini were then hand-picked sequentially and repeatedly using a 200-μl micropipette under a dissecting microscope to achieve a high purity.

RT-PCR. Expression of GLP-1R in isolated pancreatic acini, islets, or intact pancreas tissue was assessed by RT-PCR as previously described (37). Total RNA was isolated from the pancreas using TRIzol reagent (Invitrogen) and from isolated pancreatic acini and islets using an RNeasy mini-kit (Qiagen, Valencia, CA). Purity was assessed by the ratio of optical density at 260 nm to optical density at 280 nm and agarose gel electrophoresis. cDNA was synthesized using a TaqMan RT-PCR kit (Applied Biosystems, Branchburg, NJ). One microgram of cDNA was used in each PCR. Amplification with Taq DNA polymerase from the Expand High Fidelity enzyme mix kit (Roche Diagnostics, Indianapolis, IN) was conducted using specific primers for GLP-1R [5′-GCTTGACTAGGAACCTCCATG-3′ (forward) and 5′-GTACAGGATAGCCACCATCAAG-3′ (reverse)], insulin [5′-GTCACACAGCATTTTGCCAC-3′ (forward) and 5′-ACTGTGACATCAGGACCTCTCT-3′ (reverse)], and amylase [5′-CCATACCTCTGGTGGAGCTTT-3′ (forward) and 5′-GTGCTCCAATCCAGTCTCT-3′ (reverse)]. Sequencing of the PCR products was carried out in the University of Michigan DNA Sequencing Core using ABI model 3700 sequencers.

Assessment of pancreatic morphology. Hematoxylin-eosin staining of paraffin sections was performed following standard protocol. Immunohistofluorescence was performed as described previously (17, 38). Briefly, pancreatic tissues were cryosectioned after fixation for 30 min in 4% formaldehyde (freshly prepared from paraformaldehyde) in PBS. The primary anti-amylase antibody and Oregon Green 488-phalloidin were diluted 1:1,000 and 1:50, respectively. The secondary antibody anti-rabbit Alexa 594 was diluted 1:500. ProLong Gold with 4′,6-diamidino-2-phenylindole was added to the mounting medium to counterstain nuclei. Hematoxylin-eosin-stained images were taken with a ×40 objective on an Olympus BX-51 microscope. Fluorescence images (z series, 0.5-μm optical slices) were taken with an Olympus FluoView 500 confocal microscope equipped with a ×60
Measurement of amylase secretion and cAMP levels. Freshly isolated acini were used for amylase release after preincubation at 37°C for 1 h. After incubation in DMEM plus 0.1% BSA and 0.01% soybean trypsin inhibitor, GLP-1, VIP, or different secretagogues were added at various concentrations for 30 min, the acinar suspension was centrifuged for 20 s in a microcentrifuge, and the supernatant was assayed for amylase activity using Phadebas reagent (Amersham Biosciences and Upjohn) as previously described (52, 54). The pellets were collected and lysed for measurement of DNA content using a Qubit 2.0 fluorometer and Qubit double-stranded DNA high-sensitivity assay kit (Life Technologies, Eugene, OR). Secretion is expressed as percentage of initial acinar amylase total content or as units of amylase per milligram of DNA.

cAMP generation was determined as previously described (37, 38). Isolated acini were preincubated for 30 min in phenol red-free DMEM and then in phenol red-free DMEM containing 1 mM IBMX and incubated for 3 min. Acini were then stimulated with GLP-1 (300 pM, 3 nM, and 30 nM) and VIP (10 nM) for 12 min. cAMP was extracted in absolute ethanol and measured using a cAMP colorimetric enzyme immunoassay kit according to the manufacturer’s instructions. Results are expressed as picomoles per milligram of protein.

Statistical analysis. Statistical significance was determined by Student’s t-test; *P < 0.05 was considered statistically significant.

RESULTS

GLP-1R mRNA is expressed in isolated acinar cells. Because of the unavailability of good specific GLP-1R antibodies (33), we sought to identify the expression of GLP-1R by performing RT-PCR on samples from highly purified acini. Using a combined protocol of enzymatic digestion and hand-picking to obtain pancreatic islets and acini with high purity, we isolated mouse pancreatic acini and islets. GLP-1R primers from previous publications or self-designed GLP-1R primers were tested. Only the primers indicated in MATERIALS AND METHODS generated consistent and repeatable results confirming the presence of GLP-1R mRNA. The GLP-1R PCR results from mouse pancreas, isolated islets, and acini have been confirmed by DNA sequencing using the same forward primer.
as the sequencing primer. The purity of our islet and acini preparations was established by RT-PCR using primers for insulin and amylase, respectively (Fig. 1A). GLP-1R signals were detected by RT-PCR in pure acini preparations, as well as islet and pancreas preparations, but, as we expected, expression was higher in islets (Fig. 1B). In acini from the GLP-1R KO mice, the expression of GLP-1R was not detected when the same primers were used and under the same conditions (Fig. 1C).

Acinar cell morphology and expression of digestive enzymes were unaffected by GLP-1R deficiency. Because of the importance of the GLP-1R KO mice to these studies, we evaluated the effect of lack of GLP-1R on overall pancreatic morphology and pancreatic digestive enzyme content. Loss of GLP-1R had no overall effect on pancreatic morphology as assessed using hematoxylin-eosin staining (Fig. 2, A and B). Moreover, there were no apparent differences in amylase distribution and phalloidin localization as assessed by confocal immunofluorescence (Fig. 2, C and D). Furthermore, there was no obvious difference in the expression levels of five separate digestive enzymes between the KO and WT pancreases (Fig. 3, A and B). As a control for antibody specificity, there was no signal from liver and kidney (Fig. 3C). Finally, no difference in the weight of the pancreas was observed between WT and GLP-1R KO mice (8.06 ± 0.17 and 7.96 ± 0.25 mg/kg body wt, respectively).

GLP-1 increased amylase secretion from isolated acini. To investigate the effects of GLP-1R on digestive enzyme secretion, acini were isolated from WT or GLP-1R KO mice and stimulated with various concentrations of GLP-1 or VIP. GLP-1 (30 pM–3 nM) stimulated amylase secretion from WT acini but had no significant effect in GLP-1R KO acini (Fig. 4A); VIP (100 pM–10 nM) stimulated amylase release from WT and GLP-1R KO acini was essentially identical (Fig. 4B). Similar results were obtained when amylase release was normalized to DNA content (Fig. 4, C and D). We next tested whether the effect of other secretagogues on amylase release was affected by GLP-1R deletion. Isolated acini from WT and KO mice were treated with fixed concentrations of CCK, carbachol, VIP, the calcium ionophore A23187, and the cAMP analog pCPT-cAMP. There was no difference in amylase secretion between WT and KO acini for all secretagogues, except carbachol, which induced a small, but significant, decrease in amylase secretion from KO acini (Fig. 5).

GLP-1-induced cAMP signaling pathway activation was blocked in GLP-1R KO acini. To further investigate the effects of GLP-1R deficiency on the intracellular signaling pathway, isolated WT and GLP-1R KO acini were treated with different

Fig. 4. GLP-1 induces amylase release in acini from WT, but not GLP-1R KO, mice. Freshly isolated acini were incubated with 30 pM, 100 pM, 300 pM, 3 nM, and 30 nM GLP-1 (A and C) or 100 pM, 1 nM, and 10 nM vasoactive intestinal peptide (VIP, B and D) for 30 min. Amylase release is expressed as percentage of total acinar amylase content (A and B) or released amylase per milligram DNA (C and D). Values are means ± SE from 5–13 independent experiments. *P < 0.05, vs. WT control. #P < 0.05 vs. GLP-1R KO control.
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Fig. 5. GLP-1R deficiency caused a decrease in amylase release induced by GLP-1, but not most other secretagogues. Freshly isolated acini were incubated with 30 pM cholecystokinin (CCK), 1 μM carbachol (CCh), 3 nM GLP-1, 1 nM VIP, 2 μM A23187, or 100 μM 8-(4-chlorophenylthio)-2′-O-Me-cAMP (pCPT-cAMP) for 30 min. Amylase release is expressed as percentage of total acinar amylase content. Values are means ± SE from 5–9 independent experiments. *P < 0.05.

concentrations of GLP-1 and VIP. At 30 pM and 3 nM GLP-1 caused a concentration-dependent increase in cAMP production in WT acini but had no effect in GLP-1R KO acini. However, the VIP-induced elevation of cAMP was not significantly different between WT and GLP-1R KO acini (Fig. 6). These data suggest that the GLP-1-induced increase in cAMP signaling is mediated through GLP-1R. This was further explored using an anti-phosphorylated PKA substrate antibody as a readout of cAMP signaling. This antibody can specifically detect motifs that are phosphorylated by cAMP-dependent protein kinase (31). GLP-1-activated PKA signaling was abolished in KO acini, while VIP treatment increased PKA activity in KO acini, although to a lesser extent than in WT acini (Fig. 7). The effect of GLP-1 and VIP to increase phosphorylation of specific protein bands was abolished by the PKA inhibitor H-89 (data not shown).

DISCUSSION

The well-characterized actions of GLP-1 are to stimulate insulin secretion and inhibit glucagon secretion and, thereby, participate in the regulation of glucose metabolism after meal intake. In addition, GLP-1 has been shown to also inhibit gastrointestinal motility and secretion and regulate appetite and food intake. Therefore, GLP-1 analogs have been used clinically to treat type 2 diabetes. Since a potential association between the GLP-1-based therapy and the development of pancreatitis was reported (28), a great amount of research has been performed to study the possible relationship between GLP-1 analogs and pancreatitis. Notwithstanding the possibility that these agents exhibit nonspecific toxicity within the exocrine pancreas, these studies raise questions about the physiological role of GLP-1 or GLP-1R in pancreatic acinar cells. A recent study reported the effects of GLP-1R agonists in increasing pancreatic mass, although the cellular sites of GLP-1R expression were not clear (20). Therefore, the aim of the current study was to determine the physiological role of GLP-1 in pancreatic acini unambiguously using GLP-1R KO mice. Our data showed that GLP-1 can induce amylase secretion from mouse pancreatic acini and that this effect is GLP-1R-dependent, as it was abolished in acini from GLP-1R KO mice. This secretion-inducing effect is specific through GLP-1R, and secretion stimulated by other secretagogues was not affected in KO acini, with the exception of carbachol-induced secretion. This suggests that GLP-1R may interact with the function of muscarinic acetylcholine receptors; although we have no explanation for this effect, it may warrant further study.

In earlier studies on guinea pig acini, high-affinity binding of 125I-GLP-1 ligand was demonstrated, but the molecular nature of the receptors was not identified. It is possible that GLP-1 and exendin-4 might act through VIP or other receptors. The current study shows conclusively that the action of GLP-1 on mouse acini is mediated by GLP-1R. However, we did not observe a potentiating effect of GLP-1 on the CCK-induced secretion in WT or KO acini (data not shown), in contrast to the effect of GLP-1 on guinea pig pancreatic acinar cells that was reported previously (35, 40).

The presence of GLP-1R on acini indicates that this receptor could be functional in regulating the physiological response of acinar cells. GLP-1 is secreted shortly after meal intake (24), suggesting that it could regulate digestive enzyme secretion by directly binding to GLP-1R on acini. The basal level of GLP-1 in a healthy human being is ~10 pM, and GLP-1 reaches 25–45 pM postprandially (22, 30, 46). In mice, the basal plasma GLP-1 level is ~7 pM (23), and GLP-1 reaches >30 pM postprandially (32). Thus it is possible that GLP-1 could have a direct effect on acinar cells. However, an indirect mechanism also plays a role: GLP-1 can increase insulin secretion, and insulin has been shown to potentiate zymogen release from acini (53). Other studies have demonstrated that, in vivo, GLP-1 can inhibit pancreatic secretion through an

Fig. 6. Loss of GLP-1R leads to GLP-1-induced decrease in cAMP formation. Freshly prepared acini were treated with IBMX for 3 min and then with GLP-1 or VIP for 12 min. cAMP was extracted, and levels were measured by enzyme immunoassay. Values are means ± SE from 6 independent experiments. *P < 0.05 vs. WT control. #P < 0.05 vs. GLP-1R KO control.
effect on the central nervous system and vagal outflow (26, 50). An earlier study also reported that GLP-1 inhibits pancreas function in vivo, which could be a secondary effect correlated with the gastric-emptying effect of GLP-1 (49). Thus GLP-1 can regulate pancreatic digestive enzyme secretion at multiple sites. It has been clear for some time that the major secretagogues acting on acinar cells to stimulate digestive enzyme secretion are CCK and acetylcholine, which act to increase intracellular Ca\(^{2+}\) (55). GLP-1 joins other regulators, including secretin and VIP, which can enhance this response through the cAMP/PKA signaling pathway (37, 55). It has been shown that secretagogues that elevate intracellular cAMP, e.g., secretin, can also sensitize pancreatic acini to cerulein-induced zymogen activation and injury (2, 42). Although our data indicate that GLP-1 can act on acinar cells through its specific receptor, we cannot extrapolate from our findings that GLP-1 or GLP-1R agonists can induce pancreatitis through the interaction with GLP-1R, because other similar secretagogues that can trigger the cAMP signaling pathway were not reported to induce pancreatitis. Moreover, it has been shown that increasing acinar cell cAMP can overcome the inhibition of enzyme secretion caused by a high concentration of carbachol and can eliminate acinar cell injury (6); GLP-2R signaling can enhance cell survival by inhibiting the activity of a subset of proapoptotic proteins in a PKA-dependent manner (56). A recent in vivo study showed that although GLP-1 mimetics could selectively modulate the expression of genes associated with pancreatitis, they could not alter the severity of experimental pancreatitis in WT or GLP-1R KO mice (21). The other two most recent studies on the Zucker diabetic fatty rat model of type 2 diabetes further demonstrated that prolonged exposure to exenatide or liraglutide improved glucose metabolism and animal survival without showing notable effects on the exocrine pancreas (44, 47). All these studies indicate that the mechanism of action of GLP-1 on acinar cells is complicated and that systematic research is required to elucidate the mechanism of action of GLP-1 on acinar cells.

In conclusion, we have shown that GLP-1R is expressed in pancreatic acini; GLP-1 can induce secretion from acinar cells and activation of the cAMP signaling pathway from WT, but not GLP-1R KO acini, indicating that this effect is dependent on the receptor. These data indicate that GLP-1R is present in pancreatic acini and may participate in regulating secretion of digestive enzymes, increasing pancreatic mass (20), or regulating other actions on acinar cells.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Y.H. and J.A.W. developed the concept and designed the research; Y.H., S.A.E., and K.H. performed the experiments; Y.H., S.A.E., K.H., and J.A.W. analyzed the data; Y.H., S.A.E., K.H., and J.A.W. interpreted the results of the experiments; Y.H. and S.A.E. prepared the figures; Y.H. drafted the manuscript; Y.H., S.A.E., and J.A.W. edited and revised the manuscript; Y.H., S.A.E., and J.A.W. approved the final version of the manuscript.

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