Acute activation of central GLP-1 receptors enhances hepatic insulin action and insulin secretion in high-fat-fed, insulin resistant mice

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Burmeister MA, Ferre T, Ayala JE, King EM, Holt RM, Ayala JE. Acute activation of central GLP-1 receptors enhances hepatic insulin action and insulin secretion in high-fat-fed, insulin resistant mice. Am J Physiol Endocrinol Metab 302: E344–E343, 2012. First published November 15, 2011; doi:10.1152/ajpendo.00409.2011.—Glucagon-like peptide-1 (GLP-1) receptor knockout (Glp1r−/−) mice exhibit impaired hepatic insulin action. High fat (HF)-fed Glp1r−/− mice exhibit improved, rather than the expected impaired, hepatic insulin action. This is due to decreased lipogenic gene expression and triglyceride accumulation. The present studies overcome these secondary adaptations by acutely modulating GLP-1R action in HF-fed wild-type mice. The central GLP-1R was targeted given its role as a regulator of hepatic insulin action. We hypothesized that acute inhibition of the central GLP-1R impairs hepatic insulin action beyond the effects of HF feeding. We further hypothesized that activation of the central GLP-1R improves hepatic insulin action in HF-fed mice. Insulin action was assessed in conscious, unrestrained mice using the hyperinsulinemic euglycemic clamp. Mice received intracerebroventricular (icv) infusions of artificial cerebrospinal fluid, GLP-1, or the GLP-1R antagonist exendin-9 (Ex-9) during the clamp. Intracerebroventricular Ex-9 impaired the suppression of hepatic glucose production by insulin, whereas icv GLP-1 improved it. Neither treatment affected tissue glucose uptake. Intracerebroventricular GLP-1 enhanced activation of hepatic Akt and suppressed hypothalamic AMP-activated protein kinase. Central GLP-1R activation resulted in lower hepatic triglyceride levels but did not affect muscle, white adipose tissue, or plasma triglyceride levels during hyperinsulinemia. In response to oral but not intravenous glucose challenges, activation of the central GLP-1R improved glucose tolerance. This was associated with higher insulin levels. Inhibition of the central GLP-1R had no effect on oral or intravenous glucose tolerance. These results show that inhibition of the central GLP-1R deteriorates hepatic insulin action in HF-fed mice but does not affect whole body glucose homeostasis. Contrasting this, activation of the central GLP-1R improves glucose homeostasis in HF-fed mice by increasing insulin levels and enhancing hepatic insulin action.

We have shown that a functional loss of GLP-1R expression in Glp1r-knockout (Glp1r−/−) mice impairs the suppression of hepatic glucose production (HGP) but enhances the stimulation of muscle glucose uptake (MGU) during a hyperinsulinemic euglycemic clamp in chow-fed mice (4). This proposes a novel role for the GLP-1R to reciprocally regulate hepatic and muscle insulin action independently of its ability to stimulate insulin secretion. When fed a high-fat (HF) diet to promote insulin resistance, Glp1r−/− mice exhibit enhanced insulin-stimulated MGU, as observed in chow-fed mice (3). This occurred independently of any effects on body weight or fat mass. Rather than exacerbating HF diet-induced hepatic insulin resistance, as we hypothesized based on observations in chow-fed mice, loss of GLP-1R expression actually improved hepatic insulin action (3). HF-fed Glp1r−/− mice exhibited reduced hepatic triglyceride levels. This secondary adaptation would be expected to improve hepatic insulin sensitivity and potentially mask the expected phenotype of impaired hepatic insulin action due to loss of GLP-1R expression.

The present studies circumvent potential secondary adaptations by assessing the acute actions mediated by the GLP-1R in HF-fed, insulin resistant, wild-type mice. We targeted central GLP-1Rs given the growing evidence that GLP-1Rs expressed in the hypothalamus modulate hepatic and muscle insulin action. Intracerebroventricular (icv) infusion of the GLP-1R agonist exendin-4 enhances hepatic glycogen accumulation but impairs muscle glycogen accumulation during a hyperinsulinemic hyperglycemic clamp in chow-fed mice (24). Activation of GLP-1Rs in the arcuate nucleus of the hypothalamus enhances the suppression of HGP but impairs the rate of glucose disappearance (Rd) during a hyperinsulinemic euglycemic clamp in chow-fed rats (38). These studies demonstrate a role for central GLP-1Rs to reciprocally regulate hepatic and muscle insulin action, supporting our observations in chow-fed Glp1r−/− mice. Whether these regulatory effects are preserved in the insulin-resistant state is unknown.

In gain of function studies, we hypothesized that acute activation of central GLP-1Rs would overcome the hepatic insulin resistance associated with HF feeding while reciprocally further impairing muscle insulin action. In loss of function studies, we tested the hypothesis that acute inhibition of the GLP-1R in HF-fed, already insulin-resistant mice further impairs hepatic insulin action beyond the effect of HF feeding alone. We also hypothesized that acute inhibition of the GLP-1R would reciprocally enhance insulin-stimulated MGU in HF-fed mice. The role of central GLP-1Rs on the regulation of glucose disposal in response to physiological oral glucose intake was also explored.

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**MATERIALS AND METHODS**

**Mouse maintenance.** All procedures performed were approved by the Animal Care and Use Committee at the Sanford-Burnham Medical Research Institute at Lake Nona. Male C57BL/6J mice were fed a HF diet (Research Diets D12492) composed of 60, 20, and 20 kcal/g of fat, carbohydrate, and protein, respectively, for 12 wk starting at 6 wk of age. All experiments were performed on mice at 4–5 mo of age. Mice were maintained on a standard light-dark cycle (0600–1800 light).

**Surgical procedures.** Catheters were implanted in the left common carotid artery and right jugular vein for sampling and infusions, respectively, as described previously (5, 6, 9), except that surgeries were performed under inhaled isoflurane anesthesia. Intracerebroventricular cannulae were implanted using a stereotaxic apparatus (David Kopf Instruments). The skull was exposed by an incision and leveled between lambda and bregma. Cannulae (Plastics One) were implanted to target the lateral cerebral ventricle at the following coordinates: 0.3 mm caudal, 1.0 mm from midline, and 3.2 mm ventral. A guide cannula was inserted into the brain t = 60 min prior to the beginning of the infusion. Blood samples were obtained via the arterial burr holes in the skull using a drill. The guide cannula was fixed into position by two jeweler’s screws (also inserted via burr holes in the skull) and cranioplast cement. Verification of cannula position in the lateral cerebroventricle was made by observing spontaneous flow of cerebrospinal fluid from the tip of the cannula after removal of the obturator. Animals were individually housed after surgery and allowed to recover for 5 days, during which time body weight was recorded daily. Cannula placement in the lateral cerebroventricle was reverified by observing drinking behavior following administration of angiotensin on day 3 of recovery. Mice that did not return to within 15% of presurgery weight or lost catheter patency were excluded.

**Intracerebroventricular infusions.** Artificial cerebrospinal fluid (ACSF; Harvard Apparatus) was administered as vehicle. GLP-1 (3–39) amide (Acro) and the GLP-1 receptor agonist exendin (9–39) (Tocris) were infused at a rate of 0.01 µg/min.

**Hyperinsulinemic euglycemic clamps.** Insulin clamps were performed on 5-h-fasted mice (2, 4–6). A 2.5-µCi bolus of [3-3H]glucose was given at t = −90 min prior to the beginning of the insulin infusion, followed by a 0.05 µCi/min infusion for 90 min. Intracerebroventricular infusions began at t = 60 min prior to the beginning of the insulin infusion. Blood samples were obtained via the arterial catheter (2, 4–6). Basal glucose-specific activity and insulin levels were determined from blood samples at t = −15 and −5 min. The clamp was begun at t = 0 min with a continuous infusion of human insulin (Humulin R) at a rate of 4 mU/kg·min−1. The [3-3H]glucose infusion was increased to 0.15 µCi/min for the remainder of the experiment. Euglycemia (−9 mmol/l) was maintained by measuring blood glucose every 10 min starting at t = 0 min and infusing 50% dextrose as necessary. Additional blood was taken at t = 80, 90, 100, 110, and 120 min and processed to determine plasma [3-3H]glucose.

Clamp insulin levels were determined from samples at t = 120 min. A 12-µCi bolus of 2-14C deoxyglucose (2-14C DGP) was given at t = 120 min. Blood samples were obtained at t = 122, 135, 145, and 155 min and processed to determine plasma 2-14C DGP. Mice received saline-washed erythrocytes from donors beginning at t = 0 min and continuously throughout the clamp at a rate of 5.5 µL/min to prevent a fall of >5% hematocrit. At t = 155 min, mice were anesthetized with pentobarbital sodium. The soleus, gastrocnemius, superficial vastus lateralis, liver, diaphragm, heart, hypothalamus, and brain were excised, immediately frozen, and stored at −80°C.

**Tolerance tests and intravenous insulin dosing.** Oral and intravenous (iv) glucose tolerance tests and intravenous insulin injections were performed on 5-h-fasted conscious mice. Intracerebroventricular infusions began at t = −60 min prior to glucose or insulin administration. Basal glucose, insulin, and C-peptide levels were obtained at t = −5 min. At t = 0 min, a bolus of glucose (1 g/kg) was administered either orally or via the jugular vein catheter for oral and intravenous glucose tolerance tests, respectively. Blood samples were taken at t = 15, 30, 45, 60, and 90 min for blood glucose. Additional blood was taken at t = 15, 45, and 90 min for measurement of insulin and at t = 15 min for measurement of C-peptide. For iv insulin injections, a 0.5 U/kg bolus (Humulin R; Eli Lilly) was administered via the jugular vein catheter. Blood samples were taken at 15 min for the measurement of insulin and glucose, and the animals were euthanized at 30 min for tissue collection.

**Processing of plasma and tissue samples.** Plasma insulin (Merckodia) and C-peptide (ALPCO) were determined by ELISA. Plasma [3-3H]glucose and 2-14C DGP were determined from deproteinized samples, as described previously (2, 5). Tissue 2-14C DGP-6-phosphate (2-14C DGPt) radioactivity was determined as described previously (2, 5). Briefly, tissues were homogenized in 0.5% perchloric acid, and homogenates were centrifuged and neutralized with KOH. One aliquot was counted directly to determine 2-14C DGP and 2-14C DGP radioactivity. A second aliquot was treated with Ba(OH)2 and ZnSO4 to remove 2-14C DGP and any tracer incorporated into glycogen, and then it was counted to determine 2-14C DGP radioactivity. 2-14C DGP is the difference between the two aliquots. The accumulation of 2-14C DGP was normalized to tissue weight. Plasma nonesterified fatty acid content was measured by an acyl-CoA synthase and oxidase colorimetric assay (Wako). Tissue triglycerides were determined by extracting total lipids from liver, gastrocnemius muscle, and epididymal white adipose tissue with chloroform-methanol (2:1, vol/vol), as described previously (11). Plasma and tissue triglycerides were quantified spectrophotometrically using an enzymatic assay (Stanbio).

**Protein immunoblotts.** Whole cell extracts from liver and hypothalamus were obtained by homogenizing tissue (40–80 mg for liver and entire hypothalamus) in 10 µL/mg tissue extraction buffer (50 mM Tris, 1 mM EDTA, 1 mM EGTA, 10% glycerol, 1% Triton X-100, pH 7.5) supplemented with protease and phosphatase inhibitor cocktails (Sigma). Homogenates were centrifuged (20 min, 4,500 g, 4°C), pellets were discarded, and supernatants were retained for protein determination. Protein content was determined using a Bradford protein assay kit (Pierce). Whole cell (20–100 µg) extracts were separated on 4–12% Bis-Tris SDS-PAGE gels (Invitrogen), followed by electrophoretic transfer to nitrocellulose membranes. Primary antibodies were incubated with the membranes overnight at 4°C. Secondary antibodies were incubated at room temperature for 1 h. Imaging and densitometry were performed using either the Odyssey imaging system (Li-Cor) or enhanced chemiluminescence (Pierce). Antibodies for Akt, phosphorylated Akt (Ser173), AMP-activated protein kinase (AMPK), and phosphorylated AMPK (Thr172) and β-actin were from Cell Signaling Technology.

**Calculations.** Whole body glucose appearance (Ra) and disappearance (Rd) were determined using Steele non-steady-state equations (13, 43). Endogenous glucose rate of appearance (Endo Ra) was determined by subtracting the glucose infusion rate (GIR) from total Ra. Glucose metabolic index (Rm) was calculated as described previously (16, 26) using the following equation:

\[ R_m = \frac{\text{AUC 2-14C DGP tissue} \times \text{[arterial glucose]}}{\text{2-14C DGP tissue} / \text{AUC 2-14C DGP plasma}} \]

where 2-14C DGPtissue is the 2-14C DGP radioactivity in the tissue (in pmol/g), AUC 2-14C DGPplasma is the area under the curve for plasma 2-14C DGP disappearance (in pmol-min·ml−1), and [arterial glucose] is the average arterial plasma glucose (in mmol/l) from t = 2 to 35 min following the bolus of 2-14C DGP.

**Statistical analysis.** Data are presented as means ± SE. Differences between groups were determined by one-way ANOVA followed by Neuman-Keuls Multiple Comparison post hoc tests or by two-tailed t-test as appropriate. The significance level was P < 0.05.

**RESULTS**

**Baseline parameters.** Baseline parameters for clamp studies are shown in Table 1. Body weights were not different.
GLP-1 as a GLP-1 receptor (GLP-1R) agonist (4) and glucose infusion (5) in conscious, unrestrained mice to assess the ability of insulin to maintain euglycemia (Fig. 1B). Clamp insulin levels were also not different between groups (Table 1).

Suppression of Endo Ra by insulin was significantly enhanced in mice receiving icv GLP-1 (Fig. 2A). Contrasting this, central Ex-9 infusion impaired the suppression of Endo Ra by insulin (Fig. 2A). Activation of central GLP-1Rs led to a slight decrease in the ability of insulin to stimulate Rd (Fig. 2B). The opposite was observed in response to icv Ex-9 infusions, which resulted in an improvement in the ability of insulin to stimulate Rd (Fig. 2B). Tissue Ra in various skeletal muscles (Fig. 3, A–C) as well as in heart and brain (Fig. 3, D and E) was not.

Fig. 1. Arterial glucose (A) and glucose infusion (B) during insulin clamps in high-fat (HF)-fed C57Bl/6 mice receiving intracerebroventricular (icv) infusions of artificial cerebrospinal fluid (ACSF) as vehicle (∇), glucagon-like peptide-1 (GLP-1) as a GLP-1R agonist (○), or exendin-(9–39) (Ex-9) as a GLP-1R antagonist (gray triangles). Data are shown as means ± SE for 7 mice/group.

Fig. 2. Endogenous glucose rate of appearance (Endo Ra; A) and whole body glucose disappearance (Rd; B) in HF-fed C57Bl/6 mice during insulin clamps. A: basal (open bars) and clamp (gray bars) Endo Ra values are shown for each icv treatment group. A, inset: %suppression of Endo Ra from basal to clamp values in mice receiving icv ACSF (black bar), GLP-1 (open bar), or Ex-9 (gray bar). B: basal (open bars) and clamp (gray bars) Rd values are shown for each icv treatment group. B, inset: %stimulation of Rd from basal to clamp values in mice receiving icv ACSF (black bar), GLP-1 (open bar), or Ex-9 (gray bar). Data are shown as means ± SE for 7 mice/group.

### Table 1. Basal (5-h-fasted) and insulin clamp characteristics in male mice

<table>
<thead>
<tr>
<th>Intracerebroventricular Infusion</th>
<th>ACSF</th>
<th>GLP-1</th>
<th>Ex9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, g</td>
<td>40.0 ± 1.2</td>
<td>39.4 ± 1.2</td>
<td>39.1 ± 0.8</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>8.2 ± 0.5</td>
<td>8.4 ± 0.3</td>
<td>9.4 ± 0.7</td>
</tr>
<tr>
<td>Clamp</td>
<td>8.9 ± 0.2</td>
<td>8.4 ± 0.1</td>
<td>9.3 ± 0.6</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>506 ± 63</td>
<td>827 ± 167*</td>
<td>254 ± 43*</td>
</tr>
<tr>
<td>NEFA, μmol/l</td>
<td>1,220 ± 129</td>
<td>1,522 ± 203</td>
<td>1,313 ± 282</td>
</tr>
<tr>
<td>Plasma triglycerides, mg/dl</td>
<td>10.4 ± 0.6</td>
<td>12.2 ± 1.8</td>
<td>10.1 ± 1.6</td>
</tr>
<tr>
<td>Clamp</td>
<td>4.3 ± 0.3</td>
<td>4.3 ± 0.5</td>
<td>3.8 ± 0.8</td>
</tr>
<tr>
<td>NEFA, μmol/l</td>
<td>50.2 ± 5.6</td>
<td>48.2 ± 12.8</td>
<td>35.7 ± 11.6</td>
</tr>
<tr>
<td>Clamp</td>
<td>28.3 ± 5.3</td>
<td>35.3 ± 4.5</td>
<td>25.8 ± 2.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7. ACSF, artificial cerebrospinal fluid; GLP-1, glucagon-like peptide-1; Ex9, exendin-9; NEFA, nonesterified fatty acids. Basal glucose levels are from samples obtained at t = −15 and −5 min prior to the insulin clamp. Basal insulin levels are from plasma samples obtained at t = −5 min prior to the insulin clamp. Basal NEFA and triglyceride levels are from plasma samples obtained at t = −5 min. Clamp arterial glucose represents average values over t = 80–120 min of the insulin clamp. Clamp insulin, NEFA, and triglyceride levels are from plasma samples obtained at t = 120 min of the insulin clamp. *P < 0.05 vs. ACSF.
affected by any of the treatments. Taken together, these data show that modulation of the central GLP-1R results in reciprocal effects on glucose Endo Ra and Rd. Furthermore, these data indicate a role for the central GLP-1R in the regulation of hepatic but not muscle insulin action in the context of HF diet-induced insulin resistance.

Since insulin action can be influenced at the tissue level by lipid accumulation, we measured triglyceride levels in various organs at the end of the clamp. Hepatic triglyceride levels were significantly lower in mice receiving icv GLP-1 (Fig. 4A). This decrease in triglyceride levels was not observed in mice receiving icv Ex-9. Neither of the treatments affected muscle or adipose tissue triglyceride levels (Fig. 4B and C). Plasma nonesterified fatty acids and triglyceride levels were also not different between treatment groups (Table 1).

Hepatic insulin signaling proteins. To determine whether the effects of modulating central GLP-1Rs on hepatic insulin action are reflected by changes in insulin-signaling proteins, we assessed activation of hepatic Akt. The ratio of phosphor-

ulated to total Akt, a marker of Akt activation, was not affected by icv infusions of either GLP-1 or Ex-9 during the insulin clamps (Fig. 5A). We next assessed hepatic Akt activation in response to an acute iv dose of insulin. When an acute dose of insulin was given, Akt activation was greater in livers from mice that received icv GLP-1 infusions compared with ACSF infusions (Fig. 5B). Interestingly, icv infusions of Ex-9 also increased hepatic Akt activation compared with ACSF. There were no differences in circulating insulin levels between the treatment groups (ACSF = 547 ± 29; GLP-1 = 556 ± 28; Ex-9 = 520 ± 44 pmol/l).

Hypothalamic signaling. The downstream targets of GLP-1R activation in the hypothalamus are not well characterized. Hypothalamic AMPK has been shown to be inhibited by central GLP-1R activation (41). Since inhibition of hypothalamic AMPK has been shown to enhance the suppression of Endo Ra by insulin (48), we assessed the effect of activating and inhibiting the central GLP-1R on hypothalamic AMPK during insulin clamps. As shown in Fig. 6A, neither icv GLP-1 nor Ex-9 had any effect on the phosphorylation of hypotha-

lamic AMPK, a marker of its covalent activation. These results show that in the context of an insulin clamp the effect of GLP-1R activation to suppress hypothalamic AMPK is impaired in HF-fed mice. We then assessed hypothalamic AMPK activation in response to an acute dose of insulin. Under these conditions, icv GLP-1 did inhibit hypothalamic AMPK phosphorylation (Fig. 6B). Contrasting this, icv Ex-9 had no effect on hypothalamic AMPK activation.

Glucose tolerance. To assess the effect of modulating central GLP-1R activation on the response to a physiological entry of glucose, oral glucose tolerance tests were performed on mice receiving icv infusions of GLP-1 or Ex-9. As shown in Fig. 7A, acute activation of central GLP-1Rs reduced the glucose excursion 15 min after an oral glucose bolus. This was accompanied by a greater increase in insulin levels (Fig. 7B). There was a tendency for C-peptide levels to be higher in mice
receiving icv GLP-1 (Fig. 7B, inset). This effect of central GLP-1R activation was unique to oral glucose delivery. In response to an iv glucose bolus, icv infusion of GLP-1 had no effect on glucose tolerance (Fig. 7C) or insulin levels (Fig. 7D). Blockade of the central GLP-1R with icv Ex-9 had no effect on oral glucose tolerance or on insulin levels (Fig. 7A and B). Central infusion of Ex-9 also had no effect on iv glucose tolerance (Fig. 7C) or insulin levels (Fig. 7D). These results demonstrate that activation of central GLP-1Rs reduces glucose excursions following the entry of glucose via the gastrointestinal tract.

DISCUSSION

Studies from our laboratory using Glp1r−/− mice have extended the known glucoregulatory effects mediated by the GLP-1R to show that this receptor regulates insulin action in both chow-fed and HF-fed insulin-resistant mice (3, 4). A caveat in interpreting our results, particularly in HF-fed mice, is the possibility that observed phenotypes may be due to secondary effects associated with the chronic loss of functional GLP-1R expression. The present studies overcome such caveats by acutely modulating GLP-1R activation in HF-fed, insulin-resistant mice. We focused on central GLP-1Rs given the growing evidence that this population of receptors plays a role in the regulation of peripheral insulin action (10, 23, 24, 34, 38). We show that central infusion of GLP-1 enhances hepatic insulin action in HF-fed mice. This was associated with increased activation of hepatic Akt, a marker of insulin signaling and decreased triglyceride levels. In response to an oral glucose challenge, activation of central GLP-1Rs improves glucose tolerance, and this is associated with increased insulin levels. These effects on insulin levels and glucose tolerance were specific to oral glucose entry since activation of central GLP-1Rs had no effect during an intravenous glucose challenge. Inhibition of central GLP-1Rs impaired insulin-mediated suppression of hepatic glucose production. Interestingly, this was not associated with impaired hepatic insulin signaling. Neither activation nor inhibition of central GLP-1Rs had a significant effect on muscle glucose uptake. Taken together, these studies demonstrate that acute activation of central GLP-1Rs exerts beneficial glucoregulatory effects in the context of HF diet-induced insulin resistance by enhancing a glucose-stimulated rise in insulin levels and improving hepatic insulin action.
Our finding that acute activation of central GLP-1Rs enhances hepatic insulin action in HF-fed mice supports previous observations made in insulin-sensitive rodent models. Acute activation of central GLP-1Rs enhances hepatic glucose accumulation during hyperinsulminemic hyperglycemic clamps in chow-fed mice (24). In chow-fed rats, acute infusion of GLP-1 into the arcuate nucleus of the hypothalamus enhances the suppression of HGP by insulin during hyperinsulminemic euglycemic clamps (38). The studies presented here demonstrate that central GLP-1R activation can also enhance hepatic insulin action in HF-fed, insulin-resistant mice. Although not an acute study, Parlevliet et al. (34) recently showed that 2 wk of peripheral GLP-1 treatment enhances the suppression of HGP by insulin during hyperinsulminemic euglycemic clamps in HF-fed mice. This effect was blocked by concurrent icv infusion of Ex-9, suggesting that the main effect of the peripheral GLP-1 infusions was mediated via central GLP-1Rs. Unlike the positive effect exerted by central GLP-1 infusions on hepatic insulin action, acute studies in chow-fed rodents show that activation of central GLP-1Rs exerts an inhibitory effect of tissue glucose uptake. MGU and muscle glycogen accumulation are impaired in chow-fed mice receiving icv infusions of the GLP-1R agonist exendin-4 (24). Glucose Rd is also lower in rats receiving arcuate nucleus infusions of GLP-1 (38). In the present studies, acute activation of central GLP-1Rs has no significant effect on MGU in HF-fed mice. Thus, whereas central GLP-1R activation exerts effects on HGP in HF-fed mice, the inhibitory effect of central GLP-1R activation on MGU is not observed. It is possible that the impairment of insulin-stimulated MGU due to HF feeding cannot be exacerbated further. It is also possible that central GLP-1Rs regulate HGP and MGU via distinct mechanisms and/or via distinct anatomic locations. In support of the latter possibility, third-ventricle GLP-1 infusions have no effect on the ability of insulin to suppress HGP but impair the ability of insulin to stimulate glucose Rd during insulin clamps in rats (38). This contrasts the ability of GLP-1 infusions directly into the arcuate nucleus to both enhance the suppression of HGP and impair the stimulation of Rd (38). Identifying the distinct mechanisms or anatomic locations by which central GLP-1 action regulates HGP and MGU merits further study.

In loss-of-function studies, we show that acute inhibition of the central GLP-1R impairs the ability of insulin to suppress HGP. This suggests that there is sufficient activation of central GLP-1Rs such that inhibition of these receptors can precipitate a physiological effect on hepatic insulin action. It has been reported that brain stem proglucagon gene expression is up-regulated in response to HF feeding (23). This would be predicted to increase GLP-1 production in brain stem neurons that innervate GLP-1R-expressing hypothalamic neurons (20, 27, 28, 30). Our results suggest that this potentially increased production of GLP-1, and subsequent activation of central GLP-1Rs may be an attempt to counter the effects of HF feeding to increase HGP. Unlike the results obtained from clamp studies, antagonism of the central GLP-1R with Ex-9 had no obvious phenotypic effect on glucose levels during glucose tolerance tests. This could be explained by the offsetting effects of icv-infused Ex-9 to inhibit hepatic insulin action but enhance the rate of glucose disappearance. The ability to isolate tissue-specific effects highlights one of the key advantages of using isotopic tracers during clamp studies over glucose tolerance tests. Indeed, if one were to focus only on the glucose infusion rate data from the clamp studies, one might also draw the conclusion that icv Ex-9 has no effect on glucose metabolism. Another viable explanation is that HF feeding precipitates a resistance to GLP-1 much like it precipitates a resistance to insulin. In support of this, it has been shown recently that HF feeding impairs the anorectic effects of GLP-1R activation in rats (47). Thus, in the context of GLP-1 resistance, inhibition of central GLP-1Rs would have no further effect. Unlike the effects on HGP, acute inhibition of central GLP-1Rs had no effect on MGU, although it did have
a slight effect to enhance glucose Rd. Taken together, the fact that acute modulation of central GLP-1Rs exerts control over both HGP and glucose disposal in chow-fed rodents but only on HGP in HF-fed rodents further supports the hypothesis that central GLP-1Rs regulate HGP and MGU via distinct mechanisms.

It is important to note that icv infusions of GLP-1 and Ex-9 resulted in increased and decreased fasting insulin levels, respectively, during the clamp studies. Modulation of the central GLP-1R has been shown to regulate pancreatic hormone secretion in mice, although this was in response to hyperglycemia (24). Alternatively, we cannot exclude the possibility that central infusions spilled over into the periphery. Because of the large volume of blood necessary to measure GLP-1, we were unable to directly measure plasma GLP-1 levels. Nevertheless, although fasting insulin levels were affected by modulation of the central GLP-1R, Endo Rα and Rα were not. With regard to hepatic effects, this would suggest that activation of central GLP-1Rs impairs hepatic insulin action under fasting conditions but paradoxically enhances hepatic insulin action during clamp conditions. Similarly, these observations suggest that antagonism of the central GLP-1R enhances fasting hepatic insulin action but impairs hepatic insulin action during clamp conditions. One possible explanation for this apparent paradox is that the rightward shift in the insulin dose-response curve in insulin-resistant states is such that at low, fasting insulin levels changes in insulin concentration do not have an appreciable effect on Endo Rα. Only at higher, clamp insulin levels is the relationship between insulin and Endo Rα linear. Another possible explanation is that the effects of modulating the GLP-1R are evident only during conditions of stimulated metabolic flux. This would be analogous to the observation that the incretin effect mediated by GLP-1 is observed only under hyperglycemic conditions. If activation of the central GLP-1R enhances hepatic insulin action, which we propose based on our present findings, then it makes sense for this effect to be absent during a fast when hepatic insulin action should be blunted. This proposed ability of the GLP-1R to “sense” metabolic flux is intriguing and merits further study. We also cannot exclude the possibility that central GLP-1R activation stimulates basal Endo Rα even as it raises fasting insulin levels due to known stimulatory effects of central GLP-1R activation on stress hormones (17, 22, 45). Interestingly, the phenotype observed in icv Ex-9-infused mice is identical to the response associated with overnight-fasted and anesthetized mice compared with 5-h-fasted and conscious mice used in our studies and those of Knauf et al. (23). We have shown that these procedural differences affect results obtained from insulin clamp experiments (6, 7). In the present studies, glucose was clamped at levels isoglycemic with fasting glucose for HF-fed, insulin-resistant mice (~9 mmol/l). Parlevliet et al. (34) clamped glucose at a lower level (~5 mmol/l) due to the fact that overnight fasting results in lower fasting glucose. It is also possible that blocking the central GLP-1R for 2 wk is not sufficient to achieve beneficial effects on peripheral insulin action. This would further support the hypothesis that effects on peripheral insulin action in HF-fed Glp1r−/− mice or via inhibition of central GLP-1Rs are secondary to adaptations occurring in response to prolonged (i.e., >2 wk) loss of GLP-1R action. Our studies presented here circumvent this possibility and to the best of our knowledge are the first to show a direct, acute effect of modulating central GLP-1Rs in the regulation of peripheral insulin action in HF-fed, insulin-resistant mice. The fact that both acute activation and chronic inhibition of central GLP-1Rs result in beneficial effects on peripheral glucose metabolism in HF-fed mice highlights the complexity of GLP-1 biology.

Because GLP-1 is secreted in response to oral nutrient intake, we tested the effect of acutely modulating central GLP-1Rs in response to an oral glucose challenge. Activation of central GLP-1Rs enhanced oral glucose tolerance and increased insulin levels. Increased insulin levels were due to, at least in part, enhanced insulin secretion, as determined by a slight increase in C-peptide levels in response to icv GLP-1. However, we cannot rule out a contribution of GLP-1 to also suppress insulin clearance (1, 33). This effect on glucose tolerance was unique to oral glucose intake since central GLP-1R activation had no effect on glucose tolerance or insulin levels in response to an intravenous glucose challenge. However, it is likely that the peak insulin and glucose response occurred prior to our first measurement (i.e., before the 15-min time point). Thus, central GLP-1R activation may affect the peak insulin response to an intravenous glucose challenge. As discussed previously, we also cannot rule out the possibility that central infusates spilled into the periphery. However, if centrally infused GLP-1 had spilled into the periphery, we would expect that insulin levels would have been stimulated in response to intravenous glucose. Modulation of the central GLP-1R did affect basal insulin levels in the insulin clamp experiments. Although this may suggest spillover of GLP-1 or Ex-9 into the periphery, it is also possible that the central GLP-1R can regulate pancreatic hormone secretion via a brain-to-pancreas axis. Indeed, modulation of the central GLP-1R has been shown to control pancreatic insulin secretion in a slight effect to enhance glucose Rd. Taken together, the fact that acute modulation of central GLP-1Rs exerts control over both HGP and glucose disposal in chow-fed rodents but only on HGP in HF-fed rodents further supports the hypothesis that central GLP-1Rs regulate HGP and MGU via distinct mechanisms.

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Other studies have assessed the regulation of peripheral insulin action by central GLP-1Rs in HF-fed mice. However, the approach has been to chronically, rather than acutely, activate or inhibit central GLP-1Rs. Knauf (23) et al. reported that 4 wk of central GLP-1R inhibition via chronic icv infusion of Ex-9 ameliorated HF diet-induced insulin resistance via enhanced tissue glucose uptake. This is in agreement with our model of chronic loss of GLP-1R action, the global Glp1r−/− mouse, which exhibits enhanced muscle insulin action (3).

Knauf et al. (23) did not assess hepatic insulin action in their studies, so it is not known whether chronically inhibiting central GLP-1Rs also improves hepatic insulin action, as we have shown in the Glp1r−/− mouse (3). Contrasting these findings, Parlevliet et al. (34) reported recently that 2 wk of icv Ex-9 infusion had no effect on the suppression of HGP or stimulation of glucose disappearance by insulin in HF-fed mice. Several methodological differences could explain the discrepancy in results between the studies of Parlevliet et al. (34) and those of Knauf et al. (23) and our studies on Glp1r−/− mice. Parlevliet et al. (34) conducted their insulin clamps on overnight-fasted and anesthetized mice compared with 5-h-fasted and conscious mice used in our studies and those of Knauf et al. (23). We have shown that these procedural differences affect results obtained from insulin clamp experiments (6, 7). In the present studies, glucose was clamped at levels isoglycemic with fasting glucose for HF-fed, insulin-resistant mice (~9 mmol/l). Parlevliet et al. (34) clamped glucose at a lower level (~5 mmol/l) due to the fact that overnight fasting results in lower fasting glucose. It is also possible that blocking the central GLP-1R for 2 wk is not sufficient to achieve beneficial effects on peripheral insulin action. This would further support the hypothesis that effects on peripheral insulin action in HF-fed Glp1r−/− mice or via inhibition of central GLP-1Rs are secondary to adaptations occurring in response to prolonged (i.e., >2 wk) loss of GLP-1R action. Our studies presented here circumvent this possibility and to the best of our knowledge are the first to show a direct, acute effect of modulating central GLP-1Rs in the regulation of peripheral insulin action in HF-fed, insulin-resistant mice. The fact that both acute activation and chronic inhibition of central GLP-1Rs result in beneficial effects on peripheral glucose metabolism in HF-fed mice highlights the complexity of GLP-1 biology.

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response to hyperglycemia (24). Inhibition of central GLP-1Rs, regardless of the route of glucose entry, had no effect on glucose tolerance or insulin secretion. Taken together, these data suggest that in the HF-fed state insulin levels can be increased by activating central GLP-1Rs only in combination with a gastrointestinal signal. This signal could be gut-secreted GLP-1 activating pancreatic GLP-1Rs. Obesity is associated with decreased endogenous secretion of GLP-1 from the gut (36, 46). One could hypothesize that this impaired incretin effect can be improved upon by activation of central GLP-1Rs. It is also possible that reduced endogenous GLP-1 secretion in obese states results in decreased activation of central GLP-1Rs, and exogenous central infusions rescue this defect. This latter hypothesis assumes that central GLP-1Rs are activated by gut-secreted GLP-1 and not by GLP-1 produced from the brain stem, a topic that remains unresolved.

Central infusion of GLP-1 resulted in enhanced activation of hepatic insulin signaling, as measured by Akt activation, in response to an acute insulin challenge. Interestingly, antagonism of the central GLP-1R also resulted in a modest activation of hepatic Akt. This suggests a potential disconnect between hepatic insulin signaling and insulin action in response to modulation of the central GLP-1R. In support of this, it has been shown previously that enhanced muscle glycogen accumulation in response to inhibition of central GLP-1Rs during a hyperinsulinenic hyperglycemic clamp occurs even in the absence of the muscle insulin receptor (24). An alternative explanation is that hepatic insulin action correlates with lipid accumulation. Hepatic triglyceride levels were lower in GLP-1-infused mice, whereas icv Ex-9 had no effect to lower hepatic triglycerides. Given the association between tissue lipid accumulation and insulin resistance (37), this reduction in hepatic triglycerides by central GLP-1 action would be expected to improve hepatic insulin action. A reduction in hepatic triglycerides in response to central GLP-1R activation has been reported previously (31). However, in those studies, GLP-1 was chronically infused for 2 days, and the decrease in hepatic triglycerides was attributed to the suppression of food intake associated with chronic central GLP-1R activation. In the same study, the authors also observed a decrease in adipose triglyceride levels that could not be explained by the anorectic effects of GLP-1. In our present studies, we show that acute activation of central GLP-1Rs lowers hepatic triglyceride levels independent of any effects on food intake. More recently, GLP-1R activation has been shown to reduce hepatic and hepatocyte lipid accumulation via direct effects to reduce lipogenesis and stimulate lipid oxidation (42, 44). This role for the GLP-1R to regulate tissue lipid fluxes merits further investigation.

The mechanism by which central GLP-1Rs regulate HGP is unknown. Here we postulate that hypothalamic AMPK is a mediator of this effect. This is based on the independent observations that central GLP-1R activation suppresses hypothalamic AMPK (18) and that suppression of hypothalamic AMPK reduces HGP (48). In response to an acute insulin injection, central GLP-1R activation decreased phosphorylation of hypothalamic AMPK. Inhibition of AMPK would be expected to increase cellular malonyl-CoA levels and decrease fatty acid oxidation (21). Increased hypothalamic malonyl-CoA has been associated with enhanced suppression of HGP (19, 32). Furthermore, decreased fatty acid oxidation would increase hypothalamic levels of long-chain fatty acid CoAs, resulting in activation of hypothalamic ATP-sensitive potassium (K\textsubscript{ATP}) channels (29). Activation of hypothalamic K\textsubscript{ATP} channels enhances the suppression of HGP (35). Importantly, inhibition of K\textsubscript{ATP} channels blocks the ability of centrally infused GLP-1 to enhance the suppression of HGP (38). Taken together, this proposes a model whereby activation of central GLP-1Rs inhibits AMPK, resulting in increased malonyl-CoA and long-chain fatty acid CoAs and activation of K\textsubscript{ATP} channels, all of which have been linked to enhanced suppression of HGP.

We cannot rule out the potential contributions of glucagon to the hepatic effects observed in our studies. Activation of the pancreatic GLP-1R suppresses glucagon secretion (12, 15, 25), although it is not known whether activation of the central GLP-1R can suppress glucagon secretion as well. Since insulin secretion was increased during oral glucose tolerance tests in response to central GLP-1R activation, it is feasible that central GLP-1Rs exert control on the secretion of other pancreatic hormones. It is reasonable to speculate that inhibition of central GLP-1Rs stimulates glucagon secretion, resulting in increased HGP even in the presence of insulin. Because of limitations in the blood volume of the mouse, we did not measure circulating glucagon levels in these studies. We (3) and others (8, 39, 40) have previously observed no effect on glucagon levels in Glp1r<sup>−/−</sup> mice. However, glucagon levels were measured from arterial or cardiac blood samples and may not reflect concentrations in the portal circulation that targets the liver.

Although GLP-1 actions in the brain have been recognized for many years, these have focused primarily on the regulation of food intake. The present findings add significantly to the growing body of literature showing a key role for central GLP-1 action on the regulation of peripheral glucose metabolism. We propose a model whereby activation of pancreatic GLP-1Rs stimulates the secretion of insulin and activation of central GLP-1Rs enhances hepatic insulin action. This aids in the reduction of postprandial glucose excursions and ensures the replenishment of hepatic glycogen stores that provide glucose in the postabsorptive state. The growing use of GLP-1-based therapies for the treatment of diabetes highlights the importance of dissecting the different mechanisms by which GLP-1 exerts glucoregulatory control.

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DISCLOSURES

The authors have no disclosures.

AUTHOR CONTRIBUTIONS

M.A.B. and Julio E. Ayala did the conception and design of the research; M.A.B., T.F., Jennifer E. Ayala, E.M.K., R.M.H., and Julio E. Ayala performed the experiments; M.A.B., T.F., Jennifer E. Ayala, and Julio E. Ayala analyzed the data; M.A.B. and Julio E. Ayala interpreted the results of the experiments; M.A.B., T.F., and Julio E. Ayala prepared the figures; M.A.B., T.F., Jennifer E. Ayala, and Julio E. Ayala edited and revised the manuscript; M.A.B. and Julio E. Ayala approved the final version of the manuscript; Julio E. Ayala drafted the manuscript.

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