Differential effects of oxyntomodulin and GLP-1 on glucose metabolism

Xiaobing Du,1 Jennifer R. Kosinski,1 Julie Lao,2 Xiaolan Shen,3 Aleksandr Petrov,1 Gary G. Chicchi,4 George J. Eiermann,1 and Alessandro Pocai2
1In Vivo Pharmacology, Merck Sharp & Dohme Corporation, Rahway, New Jersey; 2Diabetes and Endocrinology, Merck Sharp & Dohme Corporation, Rahway, New Jersey; 3Safety Assessment and Laboratory Animal Resources, Merck Sharp & Dohme Corporation, Rahway, New Jersey; and 4In Vitro Pharmacology, Merck Sharp & Dohme Corporation, Rahway, New Jersey

Submitted 21 March 2012; accepted in final form 17 May 2012

Du X, Kosinski JR, Lao J, Shen X, Petrov A, Chicchi GG, Eiermann GJ, Pocai A. Differential effects of oxyntomodulin and GLP-1 on glucose metabolism. Am J Physiol Endocrinol Metab 303: E265–E271, 2012. First published May 22, 2012; doi:10.1152/ajpendo.00142.2012.—Glucagon-like peptide-1 (GLP-1) and oxyntomodulin (OXM) are peptide hormones secreted postprandially from the gut that stimulate insulin secretion in a glucose-dependent manner. OXM activates both the GLP-1 receptor (GLP1R) and the glucagon receptor (GCGR). It has been suggested that OXM acutely modulates glucose metabolism solely through GLP1R agonism. Because OXM activates the GLP1R with lower affinity than GLP-1, we generated a peptide analog (Q→E, OXMQ3E) that does not exhibit glucagon receptor agonist activity but retains the same affinity as OXM for GLP1R. We compared the effects of OXM and OXMQ3E in a glucose tolerance test and, to better characterize the effect on glucose metabolism, we performed controlled infusions of OXM or OXMQ3E during a hyperglycemic clamp performed in wild-type, Glp1r−/−, and Gcgr−/− mice. Our findings show that OXM, but not OXMQ3E, activates the GCGR in vivo. Second, OXM and OXMQ3E improve glucose tolerance following an acute glucose challenge and during a hyperglycemic clamp in mice. Finally, OXM infusion during a glucose clamp reduces the glucose infusion rate (GIR) despite a simultaneous increase in insulin levels in Glp1r−/− mice, whereas OXM and OXMQ3E increase GIR to a similar extent in Gcgr−/− mice. In conclusion, activation of the GCGR seems to partially attenuate the acute beneficial effects on glucose and contributes to the insulinoformic action of oxyntomodulin.

Differential effects of oxyntomodulin and GLP-1 on glucose metabolism.
blood glucose excursion profile from $t = 0$ to $t = 60$ min was used to integrate the area under the curve (AUC) for each treatment. Percent inhibition values for each treatment were generated from the AUC data normalized to the saline-challenged controls.

Hyperglycemic clamp study in DIO mice. Nineteen DIO C57BL/6N male mice were anesthetized with xylazine and ketamine and catheterized at the right internal jugular vein 5 days before the in vivo studies. The venous catheter was used for infusion, and blood samples were collected from the tail vein. Each animal was monitored for food intake and weight gain after surgery to ensure complete recovery. Hyperglycemic clamps were performed in conscious, unrestrained, catheterized mice. Dextrose (25% D-glucose, Sigma) was infused (0.1 vs. 3.5 nmol·kg\(^{-1}\)·min\(^{-1}\)), OXMQ3E (3.5 nmol·kg\(^{-1}\)·min\(^{-1}\)), or vehicle (sterile saline) was infused. At the end of the study, mice were anesthetized with isofluorane, and blood (200 μL) was collected by cardiocentesis into EDTA-coated microtainer tubes containing DPP-4 inhibitor and aprotinin. Plasma was obtained by centrifugation at 4°C and stored at −80°C.

Plasma collections in DIO mice during vehicle, OXM, and OXMQ3E infusion. A hyperglycemic clamp replicating the previous experimental design was performed for blood collection. Fifty-two DIO C57BL/6N male mice were killed at 5, 10, 30, and 60 min (4/group) following the infusion of sterile saline, OXM (3.5 nmol·kg\(^{-1}\)·min\(^{-1}\)), or OXMQ3E (3.5 nmol·kg\(^{-1}\)·min\(^{-1}\)). Blood (200 μL) was collected by cardiocentesis into EDTA-coated microtainer tubes containing DPP-4 inhibitor and aprotinin. Plasma was obtained by centrifugation at 4°C and stored at −80°C for subsequent analysis.

**RESULTS**

Acute administration of OXM activates GCGR in vivo. We previously identified an OXM analog, OXMQ3E, which retains equivalent GLP1R agonism, has no significant GCGR agonist activity in vitro, and yet differs from native OXM by only one residue (Q→E). OXMQ3E (1.1 μmol/kg) did not change the plasma levels of β-HBA (3.1 ± 0.2 vs. 5.2 ± 0.8 vs. 2.8 ± 0.6 mg/dl, vehicle vs. OXM vs. OXMQ3E, P < 0.05; Fig. 2A). These data suggest that the elevation of plasma β-HBA is mediated by activation of the GCGR by OXM in vivo. To demonstrate that the elevation of β-HBA is mediated by activation of the GCGR, Glp1r\(^{-/-}\) and Gcgr\(^{-/-}\) mice were treated with vehicle, OXM, or OXMQ3E using the protocol described above. OXM increased β-HBA in Glp1r\(^{-/-}\) (2.0 ± 0.1 vs. 3.5 ± 0.8 vs. 2.3 ± 0.1 mg/dl, vehicle vs. OXM vs. OXMQ3E, P < 0.05; Fig. 2B) but not in Gcgr\(^{-/-}\) mice (1.5 ± 0.3 vs. 1.9 ± 0.1 vs. 2.1 ± 0.1 mg/dl, vehicle vs. OXM vs. OXMQ3E, P = NS; Fig. 2C), demonstrating that OXM stimulates liver ketogenesis via GCGR activation.

**IPGTT in lean mice.** To compare the ability of OXM and OXMQ3E to lower blood glucose excursions during an IPGTT, the peptides were injected subcutaneously in lean mice (~25 g) 10 min prior to a dextrose challenge. OXM and OXMQ3E dose-dependently lowered blood glucose levels post-challenge, as evidenced by a reduction of the AUC of glucose concentrations (AUC\(_{\text{glucose}}\):

---

**Table 1:**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>mGLP1R</th>
<th>mGCCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>OXM</td>
<td>2.5</td>
<td>6.2</td>
</tr>
<tr>
<td>OXMQ3E</td>
<td>3.9</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

---

**Fig. 1:** In vitro agonist potencies of oxyntomodulin (OXM) and OXMQ3E at glucagon-like peptide-1 receptor (GLP1R) and glucagon receptor (GCGR). A: by mutation of the 3rd residue of OXM from the neutral glutamine (Q) to the acidic residue glutamate (E). B: we obtained a peptide analog (OXMQ3E) that does not exhibit GCGR agonist activity but retains similar affinity for GLP1R (C). R, peptide; m, murine.
8,432 ± 345, P < 0.05; 225 nmol/kg: 10,068 ± 609, P < 0.05; 67 nmol/kg: 12,045 ± 939, P < 0.05; 22.5 nmol/kg: 13,282 ± 642, P = NS; 2.3 nmol/kg: 14,280 ± 1,121, P = NS; Fig. 3, A and C. AUC<sub>glucose</sub>: OXM3E, 674 nmol/kg: 7,615 ± 277, P < 0.05; 225 nmol/kg: 8,785 ± 647, P < 0.05; 67 nmol/kg: 9,110 ± 261, P < 0.05; 22.5 nmol/kg: 10,298 ± 670, P < 0.05; 225 nmol/kg: 12,045 ± 939, P < 0.05; 67 nmol/kg: 14,675 ± 737, P < 0.05; 22.5 nmol/kg: 15,237 ± 552, P = NS; 6.7 nmol/kg: 16,797 ± 1,121, P = NS; Fig. 3, A and C. AUC<sub>glucose</sub>: OXM3E at 225 nmol/kg (126% inhibition of glucose AUC<sub>0-60 min</sub>; Fig. 3C), while similar efficacy was achieved with OXM3E at 22.5 nmol/kg (127% inhibition of glucose AUC<sub>0-60 min</sub>; Fig. 3D).

**Hyperglycemic clamp in DIO mice.** To explore the effect of OXM on glucose tolerance and further characterize the kinetics of the effects of OXM and OXMQ3E on glucose metabolism, we performed hyperglycemic clamps in DIO mice (Fig. 4, A and B). During the first 60 min of the clamp (Baseline), all animals were infused with vehicle and exogenous glucose to maintain steady-state hyperglycemic levels (16 mmol/l; Fig. 4C). Vehicle, OXM, or OXMQ3E was infused intravenously in the last 60 min of the study (Treatment). Body weight, blood glucose, insulin, and FFA concentrations across groups are reported in Table 1. No significant differences in blood glucose, insulin, and FFA levels were observed in mice infused with vehicle during the baseline period of the hyperglycemic clamp (t = -60/0 min). At the end of the clamp, plasma insulin was significantly higher, whereas plasma FFA were decreased in OXM- and OXMQ3E-treated mice compared with the vehicle group (Table 1). In animals treated with OXM, a rapid and robust increase in GIR was required to maintain hyperglycemia compared with baseline and vehicle-treated mice [GIR<sub>0-60 min</sub> (average GIR for the 60 min period): 41.8 ± 7.7 vs. 7.0 ± 3.0 vs. 5.8 ± 2.9 mg·kg<sup>−1</sup>·min<sup>−1</sup>, P < 0.05, OXM vs. vehicle vs. baseline; Fig. 4D]. Treatment with OXMQ3E also increased the GIR necessary to maintain hyperglycemia (GIR<sub>0-60 min</sub>: 61.2 ± 10.1 vs. 5.9 ± 3.7 vs. 5.8 ± 2.9 mg·kg<sup>−1</sup>·min<sup>−1</sup>, OXMQ3E vs. vehicle vs. baseline, P < 0.05; Fig. 4D). No significant differences were observed for GIR in the vehicle-infused mice during the experiment (GIR<sub>-60/0 min</sub>: 6.4 ± 3.2 vs. GIR<sub>0-60 min</sub>: 5.8 ± 2.9 mg·kg<sup>−1</sup>·min<sup>−1</sup>, P = NS; Fig. 4D). The GIR was ~38% higher in the OXMQ3E-treated group relative to OXM treatment (P < 0.05), and for the latter, the glucose-lowering effect was delayed by ~10 min relative to the OXMQ3E treatment. To assess the insulinotropic effect of OXM and OXMQ3E before and during glucose infusion, a separate hyperglycemic clamp was performed in DIO mice by using the same protocol described in the previous paragraph. In the presence of steady-state hyperglycemic conditions (~16 mmol/l)}
mmol/l), both treatments increased insulin levels above the levels achieved in control (vehicle) animals at 5 min (vehicle: 8.4 ± 0.4 ng/ml) and 10 min (vehicle: 7.6 ± 0.6 ng/ml) after the start of the treatment (Fig. 5). The augmentation of insulin secretion was significantly higher in OXM- than in OXMQ3E-treated mice at 5 min (5 min: 12.8 ± 1.1 vs. 9.6 ± 0.5 ng/ml, OXM vs. OXMQ3E, P < 0.05) but not at 10 min. Thirty and sixty minutes after the beginning of the infusion, the insulin levels in OXMQ3E-treated mice declined to the levels measured in OXMQ3E- and vehicle-treated mice (P = NS; Fig. 5).

Hyperglycemic clamp in wild-type, Glp1r<sup>−/−</sup>, and Gcgr<sup>−/−</sup> mice. To further characterize the pathways involved in the effect of OXM on glucose metabolism, we performed a hyperglycemic clamp study (~16 mmol/l; Fig. 6) in lean Glp1r<sup>−/−</sup>, Gcgr<sup>−/−</sup>, and weight-matched (~28 g) wild-type mice infused with vehicle, OXM, or OXMQ3E for 60 min. Body weight, blood glucose, insulin, and FFA concentrations across groups at the end of the hyperglycemic clamp are reported in Table 2. No significant differences in blood glucose and FFA levels were observed in wild-type, Glp1r<sup>−/−</sup>, and Gcgr<sup>−/−</sup> mice infused with vehicle, OXM, or OXMQ3E. Plasma insulin was significantly higher in wild-type OXMQ3E-treated mice compared with the OXMQ3E- and vehicle-treated groups (Table 2). Glp1r<sup>−/−</sup> mice are glucose intolerant and show impaired insulin secretion (1, 23). Consistently, the integrated GIR required to maintain the hyperglycemia was significantly lower in Glp1r<sup>−/−</sup> mice than in wild-type lean controls infused with vehicle (GIR<sub>0/60 min</sub>: 13.1 ± 2.9 vs. 17.2 ± 2.9, P < 0.05; Fig. 6, D and B). OXM infusion initially lowered the GIR required to maintain the hyperglycemic levels in Glp1r<sup>−/−</sup> vs. wild-type mice (GIR<sub>0/60 min</sub>: 8.5 ± 1.9 vs. 54.0 ± 7.8, P < 0.05; Fig. 6, D and B), suggesting deterioration of insulin sensitivity. OXMQ3E increased the GIR in wild-type mice (GIR<sub>0/60 min</sub>: 76.5 ± 5.5 vs. 17.2 ± 2.9, OXMQ3E vs. vehicle; P < 0.05; Fig. 6B). No significant differences were observed between OXMQ3E- and vehicle-infused mice in Glp1r<sup>−/−</sup> mice, confirming the GLP1R-selective nature of OXMQ3E in vivo (GIR<sub>0/60 min</sub>: 13.6 ± 2.2 vs. 13.1 ± 2.9, P = NS, Fig. 6D). Gcgr<sup>−/−</sup> mice exhibit improved glucose tolerance and reduced blood glucose levels in the presence of normal plasma insulin levels. In contrast, plasma glucagon and GLP-1 levels are increased (10). Consistent with the reported improved glucose tolerance, the exogenous infusion of insulin required to maintain the hyperglycemic levels was higher in Gcgr<sup>−/−</sup> mice than in wild-type animals infused with vehicle (GIR<sub>0/60 min</sub>: 23.4 ± 2.1 vs. 17.2 ± 2.9, P < 0.05; Fig. 6F and B) despite the lower insulin levels measured at the end of the study in Gcgr<sup>−/−</sup> mice (Table 2). Treatment of Gcgr<sup>−/−</sup> mice with OXM and OXMQ3E resulted in a comparable increase in GIR (GIR<sub>0/60 min</sub>:

Table 1. Hyperglycemic clamp in DIO mice

<table>
<thead>
<tr>
<th></th>
<th>Baseline (vehicle) &lt;sub&gt;0/60 min&lt;/sub&gt;</th>
<th>Treatment &lt;sub&gt;0/60 min&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>OXM</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>45.0 ± 2.1</td>
<td>43.8 ± 1.2</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>16.3 ± 2.1</td>
<td>15.0 ± 0.8</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>8.0 ± 0.4</td>
<td>8.9 ± 0.9</td>
</tr>
<tr>
<td>FFA, mM</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.0</td>
</tr>
</tbody>
</table>

Body weight and plasma parameters before (baseline) and at the end of the hyperglycemic clamp in (DIO) mice. Data are means ± SE. OXM, oxyntomodulin; OXMQ3E, peptide analog of OXM; FFA, free fatty acids. *P < 0.05 vs. vehicle; †P < 0.05 vs. baseline.
96.4 ± 11.1 vs. 109.3 ± 6.8, P = NS; Fig. 6F). Notably, the increase in GIR in Gcgr<sup>−/−</sup> mice treated with OXM and OXMQ3E was higher than the GIR increase induced by OXMQ3E in wild-type mice (GIR<sub>0/60 min</sub>: 76.5 ± 2.9, P < 0.05; Fig. 6B).

**DISCUSSION**

OXM, a longer isoform of glucagon that is cosecreted together with GLP-1 from L-cells in the gut, is a dual GLP1R/GCGR agonist (15). OXM reduces body weight in humans and rodents (2, 6, 27) and acutely improves glucose metabolism in mice (16, 20). We (15) recently demonstrated in rodents that the chronic body weight-lowering effects of OXM involve activation of both GLP1R and GCGR. The precise receptor and signaling pathways transducing the glucoregulatory actions of OXM remain uncertain. Maida et al. (16) suggested that the acute effect of OXM on glucose is mediated solely through a GLP1R-dependent pathway. However, OXM was reported to increase hepatic glucose production and ameliorate glucose intolerance during a euglycemic-hyperinsulinemic clamp performed in DIO mice, suggesting activation of the hepatic...
Table 2. Hyperglycemic clamp in WT, Glp1r−/−, and Gcgr−/− mice

<table>
<thead>
<tr>
<th></th>
<th>WT Mice</th>
<th>Glp1r−/− Mice</th>
<th>Gcgr−/− Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle, g</td>
<td>27.7 ± 2.1</td>
<td>26.9 ± 1.2</td>
<td>26.8 ± 0.7</td>
</tr>
<tr>
<td>OXM, g</td>
<td>29.2 ± 0.7</td>
<td>28.1 ± 0.7</td>
<td>28.9 ± 1.2</td>
</tr>
<tr>
<td>OXMQ3E, g</td>
<td>27.7 ± 2.1</td>
<td>26.9 ± 1.2</td>
<td>28.1 ± 0.7</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>28.0 ± 1.1</td>
<td>27.6 ± 1.7</td>
<td>29.2 ± 1.1</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>16.9 ± 2.4</td>
<td>17.2 ± 0.9</td>
<td>16.4 ± 0.8</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>6.2 ± 0.5</td>
<td>6.8 ± 0.7*</td>
<td>5.5 ± 0.4</td>
</tr>
<tr>
<td>FFA, mM</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.3 ± 0.1</td>
</tr>
</tbody>
</table>

Body weight and plasma parameters at the end of the infusion of vehicle, OXM, or OXMQ3E. Data are means ± SE. GLP1R, glucagon-like peptide-1 receptor; GCGR, glucagon receptor. *P < 0.05 vs. vehicle.

The neutral effect observed following a single intraperitoneal half-life in vivo and is a known substrate of DPP-4 (13, 19, 28). Mice (16) may be explained by the fact that OXM has a short residence time and pharmacokinetics.

We observed that acute injection of native OXM, but not OXMQ3E, increased ketogenesis, suggesting acute in vivo activation of the GCGR. This effect was then reproduced with OXM in Glp1r−/− but not in Gcgr−/− mice, demonstrating that the increased ketogenesis is due to activation of the hepatic GCGR in vivo. Consistent with previous data reported by Maida et al. (16), acute injection of OXM improved glucose excursion during a glucose tolerance test performed in mice. However, we demonstrated that a matched-pair peptide without GCGR activity in vitro and in vivo exerted better glucose lowering at equimolar doses during an IPGTT performed in lean mice. We then controlled the glucose levels and the infusion rate of OXM and OXMQ3E in DIO mice during a hyperglycemic clamp. Confirming previous observations (20), OXM improved glucose tolerance in DIO mice as denoted by the increased GIR required in animals treated with OXM compared with vehicle-infused mice. The important observation in this study is that the matched GLP1R-selective peptide OXMQ3E improved glucose tolerance at lower doses than OXM, suggesting that activation of the GCGR may reduce the acute glucose-lowering efficacy of OXM. Finally, hyperglycemic clamps confirmed the decreased improvement in glucose tolerance in wild-type mice treated with OXM vs. wild-type mice treated with OXMQ3E and showed decreased insulin sensitivity in OXM-treated Glp1r−/− mice compared with vehicle-treated animals. These data suggest that in the absence of a functional GLP-1 receptor, controlled and matched infusions of OXM results in reduction in glucose tolerance, as shown by the reduced exogenous glucose required to maintain hyperglycemia compared with vehicle-infused Glp1r−/− mice. The neutral effect observed following a single intraperitoneal injection of OXM during an OGTT performed in Glp1r−/− mice (16) may be explained by the fact that OXM has a short half-life in vivo and is a known substrate of DPP-4 (13, 19, 28). In addition, Glp1r−/− mice are glucose intolerant and resistant to diet-induced obesity; hence, the acute glucoregulatory effect of OXM could be confounded by compensatory mechanisms associated with chronic deletion of the GLP1R. Another interesting finding of our study was the initial increase of insulin levels observed during infusion of OXM in Glp1r−/− mice. This elevation in the presence of tightly controlled hyperglycemic conditions cannot be explained by an indirect effect of glucose on the pancreas. The GCGR is expressed on rodent β-cells (18). It is possible that the stimulation of insulin secretion is the result of a direct effect of glucagon on the β-cells and/or activation of unidentified receptor(s) for OXM. Supporting a potential direct effect of glucagon on the β-cells, it has been shown that the glucose-dependent insulin secretion observed in isolated murine islets following incubation of OXM was significantly reduced but not completely abolished in the presence of the GLP1R antagonist exendin(9–39) (16). Further studies will be required to address the contribution of the insulinotropic action of glucagon on β-cells. Activation of GLP1R by glucagon [EC50 ~ 0.5 nM (22)] may confuse the interpretation of results obtained in isolated wild-type islets. To further strengthen our data, hyperglycemic clamps performed in Gcgr−/− mice confirmed that the different GIR observed between OXM and OXMQ3E in wild-type mice is abolished in the absence of a functional GCGR. Interestingly, the effect of OXM and OXMQ3E on GIR was comparable in Gcgr−/− mice but the exogenous glucose required to maintain the hyperglycemic levels was significantly higher than in wild type mice treated with OXMQ3E. These data suggest that under our experimental conditions, the reported increase in insulin sensitivity overrides the altered β-cell function of Gcgr−/− mice (24). A potential limitation in the interpretation of the present study is that deletion of a select gene in islets from knockout models can change the expression of other genes in the same family (9, 21). In our study, controlling the infusion and matching the exposures of OXM and OXMQ3E and the activation of the GLP1R, we demonstrated that OXM improves glucose metabolism despite the simultaneous activation of the GLP1R and GCGR in vivo. The glucose-lowering effect of OXM is mostly mediated by GLP1R activation, and activation of the GCGR appears to limit the acute antihyperglycemic properties of OXM.

ACKNOWLEDGMENTS

Financial support for this study was provided by Merck Sharp & Dohme Corporation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s). The authors are employed by Merck Sharp & Dohme Corp. No conflict of interest exists.

AUTHOR CONTRIBUTIONS


REFERENCES

REFERENCES


