Exogenously imposed postprandial-like rises in systemic glucose and GLP-1 do not produce an incretin effect, suggesting an indirect mechanism of GLP-1 action

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Ionut, Viorica, Idit F. Liberty, Katrin Hucking, Maya Lottati, Darko Stefanovski, Dan Zheng, and Richard N. Bergman. Exogenously imposed postprandial-like rises in systemic glucose and GLP-1 do not produce an incretin effect, suggesting an indirect mechanism of GLP-1 action. Am J Physiol Endocrinol Metab 291: E779–E785, 2006. First published May 23, 2006; doi:10.1152/ajpendo.00106.2005.—The insulinotropic intestinal hormone GLP-1 is thought to exert one of its effects by direct action on the pancreatic β-cell receptors. GLP-1 is rapidly degraded in plasma, such that only a small amount of the active form reaches the pancreas, making it questionable whether this amount is sufficient to produce a direct incretin effect. The aim of our study was to assess, in a dog model, the putative incretin action of GLP-1 acting directly on the β-cell in the context of postprandial rises in GLP-1 and glucose. Conscious dogs were fed a high-fat, high-carbohydrate meal, and insulin response was measured. We also infused systemic glucose plus GLP-1, or glucose alone, to simulate the meal test values of these variables and measured insulin response. The results were as follows: during the meal, we measured a robust insulin response (52 ± 9 to 136 ± 14 pmol/L, P < 0.05 vs. basal) with increases in portal glucose and GLP-1 but only limited increases in systemic glucose (5.3 ± 0.1 to 5.7 ± 0.1 mmol/L, P = 0.1 vs. basal) and GLP-1 (6 ± 0 to 9 ± 1 pmol/L, P = 0.5 vs. basal). Exogenous infusion of systemic glucose and GLP-1 produced a moderate increase in insulin (43 ± 5 to 84 ± 15 pmol/L, 43% of the meal insulin). However, infusion of glucose alone, without GLP-1, produced a similar insulin response (37 ± 6 to 82 ± 14 pmol/L, 53% of the meal insulin, P = 0.7 vs. glucose and GLP-1 infusion). In conclusion, in dogs with postprandial rises in systemic glucose and GLP-1, the hormone might not have a direct insulinotropic effect and could regulate glycemia via indirect, portohepatic-initiated neural mechanisms.

glucagon-like peptide-1; insulin secretion; portal; meal test; neural mechanism

EXENDIN, A PROTEIN ISOLATED FROM THE SALIVA of the Gila monster, ameliorates the effects of type 2 diabetes by acting as an agonist to the glucagon-like peptide-1 (GLP-1) receptor (9, 14). The natural ligand of this receptor, GLP-1, is an intestinal hormone released in response to food intake. GLP-1 is known to potentially contribute to glucose regulation by several mechanisms, such as 1) increasing insulin secretion, 2) decreasing glucagon secretion, 3) inhibiting gastric emptying, and 4) reducing intestinal motility. It is generally accepted that GLP-1 increases insulin secretion directly by acting on the pancreatic β-cell. GLP-1 receptors are present on islet cells, and numerous in vivo and in vitro studies (9, 17) have demonstrated increased insulin secretion in the presence of GLP-1. However, it is also known that most GLP-1 released from the intestinal L cells in the active forms GLP-1-(7–37) and GLP-1-(7–36) amide is rapidly degraded to inactive GLP-1-(9–36) amide before the arterial circulation is reached. Only a minor fraction of the secreted peptide actually reaches the pancreas in an active form (7, 16, 21). This extensive and rapid degradation of GLP-1 in blood suggests that that the amount of active GLP-1 remaining to act directly on the β-cell may be of a small enough magnitude to preclude a “classical” direct incretin effect. Although GLP-1 has been clearly shown to be insulinotropic in conditions of hyperglycemia in both humans and dogs (20, 22), many of the studies (9, 17) suggesting an incretin effect of GLP-1 were conducted at supraphysiological GLP-1 and glucose concentrations, making it difficult to assess the physiological importance of the direct effect of the peptide on the pancreas.

Systemic infusion of GLP-1 and glucose in humans, to achieve values similar to those measured after a mixed meal, produced a rise in insulin higher than the increase produced by infusion of glucose alone (37). In agreement with this result, administration of the GLP-1 receptor antagonist exendin-9–39 during an oral glucose tolerance test in humans blunted the insulin response (10). However, in another study (15), infusion of systemic GLP-1 during a mixed meal in healthy humans resulted in lower blood glucose concentration, but also lower insulin, suggesting an extrapancreatic effect of the peptide. Similarly, lower meal-related glycemic excursions were measured in healthy humans receiving a systemic GLP-1 infusion together with the meal. Again, this was not accompanied by a corresponding increase in insulin release. In fact, the integrated incremental insulin response was reduced, leading Nauck et al. (28) to conclude that the inhibition of gastric emptying predominates over the insulinotropic effect. The above-mentioned results indicate that, during meals, systemic GLP-1 decreases glycemia, but direct effects on the β-cell may not predominate. GLP-1’s incretin effect during a meal may be mediated less by direct action on the pancreatic β-cell than via an indirect, neural mechanism, with sensors situated in the portohepatic area. Indeed, intraportal administration of GLP-1 has been shown to increase electrical activity in the vagus nerve (24). Moreover, intraportal GLP-1 increased glucose-stimulated in-

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insulin secretion in rats (2). In a recent study (18), we showed that, in dogs, infusion of glucose and GLP-1 into the portal vein, at values and a pattern simulating those seen during a meal, produces lower peripheral glycemia than intraportal infusion of glucose alone. The lower systemic glycemia occurred without a corresponding increase in peripheral insulin levels and was accompanied by a counterregulatory hormonal response. Thus simulation of mixed-meal glucose and GLP-1 portal values via intraportal infusion produced an insulin-independent effect on peripheral glycemia. Our study adds to recent evidence (2, 24) regarding a portohepatic mechanism of action of GLP-1. Given the possibility that the peripheral increases in glucose and GLP-1 seen during a meal might not produce a direct incretin effect, the present study was designed to reassess the incretin role of GLP-1 acting directly on the pancreatic β-cell. Specifically, we investigated whether changes in peripheral glucose and GLP-1 that are seen during a meal are adequate to produce a direct insulinotropic effect of GLP-1. Additionally, we sought to determine whether changes in peripheral levels of glucose and GLP-1 could account for the insulin-independent glucose disappearance produced by the intraportal infusions and described in our previous study (18). If not, the failure to demonstrate a clear incretin effect would be consistent with an extrapancreatic effect of GLP-1 mediated via a portal neural reflex loop.

MATERIALS AND METHODS

Animals. Experiments were performed on 20 male mongrel dogs (29.6 ± 0.8 kg body wt) in the conscious, relaxed state. All animals were deprived of food for 12–16 h before the experiments. Dogs were housed under controlled kennel conditions (12:12-h light-dark cycle) in the University of Southern California Medical School Vivarium and were fed standard diet (26% protein, 15% fat, and 40% carbohydrate, Prolab canine diet; PMI Nutrition International, Brentwood, MO) once a day. Dogs were used for experiments only if judged to be in good health as determined by body temperature, hemocrit, regularity of food intake, and direct observation. All surgical and experimental procedures were approved by the University of Southern California Institutional Animal Care and Use Committee.

Surgical procedures. At least 1 wk before the first experiment, chronic catheters (Tygon, ID = 0.050; Norton Plastics, Akron, OH) were implanted under anesthesia induced with pentothal sodium and maintained with isoflurane. In all dogs, catheters were placed in the jugular vein (with the tip advanced into the right atrium) for sampling of mixed venous blood (referred to as “peripheral”). Additionally, in eight dogs, catheters were placed in the portal vein (4 cm upstream from the porta hepatis). All catheters were led subcutaneously to the back of the neck and exteriorized. Catheters were filled with heparinized saline (10 U/ml), coiled and capped, and then placed in a small bag protected with a stiff collar.

Experimental protocol. Twenty dogs participated in this study. A meal test was performed in eight animals. Systemic glucose and GLP-1 infusions were performed on a separate group of 12 animals.

Mixed-meal test. At ~7 AM, animals were brought to the laboratory and placed in a Pavlov sling. After basal sampling at ~20 and ~10 min, a test meal (9 ml/kg of a mixture of commercial sour cream, 17% fat; Daisy Brand) and sucrose solution (1 M) were given by oral gavage with the use of a feeding tube (8). The meal was followed by simultaneous portal and jugular vein samplings at 5, 10, 20, 30, 40, 60, 90, 120, 150, 180, 210, and 240 min. The portal and peripheral glucose concentrations measured during the meal test have been previously presented in graph form (18). In six of the eight dogs, a small dose of acepromazine (0.1 mg/kg body wt) was used during the oral gavage tube insertion. We (19) have shown that acepromazine does not influence parameters of glucose metabolism.

Systemic infusions. Two infusion protocols (glucose plus GLP-1 infusion or glucose-only infusion) were performed in each animal, on separate days, in random order. An intracatheter (BD Intracath 19 GA; Becton-Dickinson, Sandy, UT) was placed in the saphenous vein and secured. After 20 min, two basal samples were taken from the jugular vein. Infusions of either glucose and GLP-1 or glucose only were started in the saphenous vein at t = 0. Glucose (20% hydrated dextrose, anhydrous concentration 181.8 mg/ml; B Braun, Irvine, CA) was infused at a variable rate designed to achieve peripheral glucose values matching those observed during the meal test. GLP-1 was infused at a constant rate of 3 pmol·kg⁻¹·min⁻¹. In both infusion experiments, blood was sampled from the jugular vein catheters at 5, 10, 20, 30, 40, 60, 90, 120, 150, 180, 210, and 240 min.

GLP-1 used for infusions, human GLP-1 (7–36) amide, was purchased from Sigma-Aldrich (St. Louis, MO) as a lyopholized powder and dissolved in saline with 1% dog albumin to obtain a stock solution of 3 μmol/l. For each experiment, an aliquot of this stock solution was added to an infusate solution of saline with 0.5% dog albumin, such that the theoretical infusion dose would be 3 pmol·kg⁻¹·min⁻¹. The biological activity of GLP-1 was proven as follows: in a subset of four dogs, either saline or GLP-1 was infused systemically in paired experiments; 50% glucose (B Braun) was infused at 17 mg·kg⁻¹·min⁻¹ in both experiments to achieve hyperglycemia of ~14 mmol/l. Under these conditions, infusion of GLP-1 produced an insulinotropic response higher than that of saline (P = 0.02), as previously described (20). Thus, under hyperglycemic conditions, GLP-1 administered systemically did enhance insulin secretion.

Blood sampling. Samples for determination of glucose, insulin, and nonesterified fatty acids (NEFA) were collected into 1.7-ml chilled tubes coated with lithium fluoride and heparin containing 50 μl of EDTA (2% w/vol; Sigma). Samples for assay of C-peptide and glucagon were collected into tubes containing 25 μl of EDTA, 50 μl of Trasylol (10,000 KIU/ml; Serological Proteins, Kankakee, IL), and 50 μl of 0.1 mM diprotin A (Sigma). Samples for the assay of active GLP-1 were collected into tubes containing EDTA and 10 μM blood dipeptidyl peptidase IV inhibitor (Linco Research, St. Charles, MO). All samples were stored on ice until centrifugation, after which plasma was separated and stored at ~80°C until analysis. To prevent triglyceride breakdown, the NEFA samples were kept on ice and either immediately assayed or kept at ~80°C until NEFA determination.

Assays. Blood glucose concentration was determined by the glucose oxidase method using a YSI 2300 autoanalyzer (Yellow Springs Instruments, Yellow Springs, OH), and remaining plasma was stored at ~80°C for further analysis. Insulin was measured using a human insulin enzyme-linked immunonassay kit (Linco Research, St. Louis, MO). All samples were stored on ice until centrifugation, after which plasma was separated and stored at ~80°C until analysis. To prevent triglyceride breakdown, the NEFA samples were kept on ice and either immediately assayed or kept at ~80°C until NEFA determination.

Statistical analysis. Results are presented as means ± SE. Comparisons of plasma levels of variables between basal and subsequent time points within an experimental group were made using ANOVA, followed by paired t-tests to identify the significantly different time points. Comparisons between timelines for infusion experiments were done using repeated-measures ANOVA. Area under the curve (AUC) was calculated using the trapezoidal rule. All differences were considered statistically significant when P < 0.05.
peptide-1 (GLP-1) (C); and insulin (Fig. 1A). Despite this substantial increment in portal glucose, there was only a minimal and nonsignificant trend for increase in mean systemic glucose concentration, a maximum of 0.4 ± 0.1 mmol/l at 1 h (P = 0.1 vs. basal). With the meal, active GLP-1 concentration doubled in portal blood (peak: 13 ± 3 pmol/l vs. basal: 6 ± 0 pmol/l; Fig. 1B). In contrast, in peripheral blood, there was only a small trend for increasing GLP-1 (from 6 ± 0 to a maximum of 8 ± 1 pmol/l, P = 0.07). This minimal increase is presumably due to dilution of portal blood into the systemic circulation along with rapid degradation of GLP-1 in plasma and liver. The meal caused a robust tripling of portal insulin (from 121 ± 37 to peak value of 394 ± 25 pmol/l), which was mirrored in peripheral blood insulin (from 52 ± 9 to 136 ± 14 pmol/l, P < 0.001; Fig. 1C). The increase in insulin was echoed by portal and peripheral increases in C-peptide (P < 0.05; Table 1), NEFA declined significantly in portal and peripheral blood (P < 0.001), whereas glucagon did not change (Table 1).

Systemic glucose plus GLP-1 infusion. Our goal was to infuse glucose and GLP-1 systemically and attempt to simulate the peripheral levels of these compounds observed during the mixed meal. In fact, GLP-1 increased to values exceeding those measured during the meal test (from 6 ± 0 to 21 ± 4 pmol/l, P < 0.05; Fig. 2A). We measured a small increment in peripheral glucose (from 5.2 ± 0.1 to a maximum of 5.7 ± 0.2 mmol/l) that mimicked peripheral glucose during the meal test (from 5.3 ± 0.1 to a peak value of 5.7 ± 0.2 mmol/l; Fig. 2A). Despite the fact that systemic GLP-1 concentration was higher than during the meal test, the combined systemic glucose plus GLP-1 infusion resulted in an insulin increase in peripheral circulation from 43 ± 5 to a maximum of 84 ± 15 pmol/l (P < 0.01 vs. basal), far less than the insulin response of the meal. The integrated insulin AUC above basal during systemic glucose plus GLP-1 infusion was 3,279 ± 575 pmol·l⁻¹·min, amounting to only 43% of the insulin response seen during the meal test (Fig. 2C). C-peptide significantly increased in peripheral blood from 0.19 ± 0.01 to 0.27 ± 0.03 pmol/l (P < 0.05 vs. basal); there were no significant increases in glucagon, whereas NEFA were minimally suppressed (Table 2).

Systemic glucose infusion. To determine the insulinotropic effect of GLP-1 beyond that produced by glucose alone, we also performed experiments in which glucose plus saline were infused systemically. The glucose infusion rate was identical to the glucose infusion rate used in the glucose plus GLP-1 infusion protocol. When glucose was infused without GLP-1, peripheral plasma glucose values increased from 5.1 ± 0.1 to

Table 1. Portal and peripheral C-peptide, glucagon, and NEFA during the mixed meal

<table>
<thead>
<tr>
<th>C-peptide, pmol/l</th>
<th>Basal</th>
<th>20</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal</td>
<td>0.27±0.03</td>
<td>0.39±0.09</td>
<td>0.37±0.04*</td>
<td>0.35±0.06</td>
<td>0.22±0.03*</td>
<td>0.21±0.04*</td>
</tr>
<tr>
<td>Peripheral</td>
<td>0.21±0.03</td>
<td>0.27±0.03</td>
<td>0.32±0.04*</td>
<td>0.26±0.04</td>
<td>0.19±0.03</td>
<td>0.19±0.02</td>
</tr>
<tr>
<td>Glucagon, ng/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portal</td>
<td>58±16</td>
<td>73±13</td>
<td>69±15</td>
<td>67±9</td>
<td>78±25</td>
<td>63±19</td>
</tr>
<tr>
<td>Peripheral</td>
<td>49±16</td>
<td>53±7</td>
<td>58±6</td>
<td>52±3</td>
<td>54±8</td>
<td>48±7</td>
</tr>
<tr>
<td>NEFA, mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portal</td>
<td>0.57±0.10</td>
<td>0.37±0.09</td>
<td>0.27±0.07*</td>
<td>0.18±0.02*</td>
<td>0.33±0.04</td>
<td>0.42±0.09</td>
</tr>
<tr>
<td>Peripheral</td>
<td>0.56±0.10</td>
<td>0.34±0.08</td>
<td>0.22±0.03*</td>
<td>0.16±0.02*</td>
<td>0.30±0.05</td>
<td>0.48±0.09</td>
</tr>
</tbody>
</table>

Values represent means ± SE. NEFA, nonesterified fatty acids. *Significantly different from basal, P < 0.05.
a peak of 5.7 ± 0.2 mmol/l (Fig. 2A). As expected with intravenous glucose infusion, there were no changes in active GLP-1, which remained constant at basal (4 ± 1 pmol/l; Fig. 2B). Importantly, the increase in insulin produced by systemic glucose infusion was very similar to that determined by systemic glucose plus GLP-1 infusion (from 37 ± 6 to 82 ± 14 pmol/l, \( P = 0.66 \); Fig. 2C). In fact, the integrated AUC above basal was 3,997 ± 886 pmol·l\(^{-1}\)·min, amounting to 53% of the meal test insulin response. This area was not different from that produced by glucose plus GLP-1 infusion (\( P = 0.35 \)). C-peptide values increased to a similar magnitude (\( P = 0.86 \) vs. glucose alone) (Table 2). There was no significant increase in glucagon, whereas NEFA were suppressed (Table 2). Thus appearance of active GLP-1 in peripheral plasma similar to that seen during a meal test had no significant effect on insulin release compared with the effect of systemic glucose alone.

Table 2. C-peptide, glucagon, and NEFA during systemic glucose plus GLP-1 infusion and during systemic glucose-only infusion

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>C-peptide, nmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ GLP-1</td>
<td>0.19±0.01</td>
<td>0.24±0.02</td>
</tr>
<tr>
<td>− GLP-1</td>
<td>0.16±0.02</td>
<td>0.24±0.02</td>
</tr>
<tr>
<td>Glucagon, ng/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ GLP-1</td>
<td>36±4</td>
<td>31±5</td>
</tr>
<tr>
<td>− GLP-1</td>
<td>37±5</td>
<td>37±4</td>
</tr>
<tr>
<td>NEFA, mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ GLP-1</td>
<td>0.50±0.05</td>
<td>0.49±0.06</td>
</tr>
<tr>
<td>− GLP-1</td>
<td>0.58±0.05</td>
<td>0.48±0.05</td>
</tr>
</tbody>
</table>

Values represent means ± SE. GLP-1, glucagon-like peptide-1.
Presumably, this failure of GLP-1 per se to enhance insulin is likely related to the very modest peripheral increase in glycemia, which was observed during a mixed meal in the dog.

DISCUSSION

GLP-1 has a potent effect to improve glucose tolerance, and exendin-4, a GLP-1 analog isolated from the saliva of the Gila monster, has been approved for human use to ameliorate symptoms of type 2 diabetes (9, 14). However, the mechanisms of action of these peptides remain unclear. The mechanisms appear to include slowing of gastric emptying and reduction in glucagon as well as an insulin-independent effect on glucose disposal that is mediated via receptors in the portal vein (6, 18, 28). However, the classically proposed effect of GLP-1 to enhance glucose disappearance is as an incretin (9, 14). GLP-1 has been shown (22, 23) to augment the response of the pancreatic β-cells to glucose stimulation. Thus it has been considered that GLP-1 acts through the plentiful specific receptors in the pancreatic islets to directly augment the response to glucose.

To test this direct action of GLP-1 as a putative incretin, we examined in dogs whether changes in peripheral glucose and GLP-1 seen during a meal are sufficient to account for a direct incretin effect of GLP-1 appearing in peripheral circulation. We infused glucose and GLP-1 systemically to achieve increments similar to those measured during administration of the meal. The GLP-1 levels we achieved are similar to other reports in the dog model. For example, Freyse et al. (12) infused active GLP-1 at 10 pmol·kg⁻¹·min⁻¹, which resulted in peripheral active GLP-1 increases from ~5 to 20 pmol/l, a fourfold increase, proportional to our results. Similarly, Nishizawa et al. (29) reported a fourfold increase in peripheral GLP-1, from 12 to 51 pmol/l, during a constant infusion of GLP-1 at 5 pmol·kg⁻¹·min⁻¹ in the dog model.

In the present studies, replication of a meal test’s peripheral glucose, together with elevating systemic GLP-1 levels considerably higher than those observed after a meal, produced an insulin response that amounted to only ~40% of the insulin response seen during the meal. Importantly, infusion of glucose without GLP-1 augmentation produced a similar insulin response despite no increase from basal in GLP-1. Thus the presence of GLP-1 in the peripheral circulation did not augment glucose-stimulated insulin response; i.e., systemic GLP-1 did not participate in the incretin effect in the normal dog model under conditions of minimal increases in peripheral glycemia.

The failure of GLP-1 to augment the insulin secretory response in our present experimental setting could be seen as contradicting earlier results (22, 37) demonstrating that systemic infusion of GLP-1 has an insulinotropic effect beyond that produced by glucose alone. However, there are several potential explanations for the present result. One aspect that differentiates our study from other studies (22, 37) is the relatively small increase in peripheral glucose during the meal in our dog model, a pattern that was subsequently mimicked in the infusion experiments. However, far from being uncommon, postprandial glucose regulation without a substantial increase in systemic glucose is characteristic of normal dogs (8, 36). The failure of glucose to increase is due to a perfect match between glucose appearance and glucose disappearance, and it demonstrates the exquisite glucoregulation of the dog model. In normal and insulin-sensitive human volunteers, modest increases of only ~0.6 mmol/l in glucose have been reported (13, 34) during a meal. There is no consensus regarding the insulinotropic effect of GLP-1 during a small postprandial systemic glucose increase. Infusion of physiological doses of GLP-1 in healthy volunteers did not produce a significant insulin response in the absence of a rise in glucose in some studies (27, 33), although other researchers (37) found that a similar dose of GLP-1 was insulinotropic at fasting and postprandial glucose levels. One limitation of many studies (9, 17) that show an insulinotropic effect for GLP-1 is the employment of nonphysiological constant infusions of glucose, at rates designed to produce the maximum glycemia achieved during a meal, that are maintained for a longer period than in the normal pattern seen during a meal. It is therefore possible that, in a physiological situation such as the mixed meal in the dog model, the glycemic threshold for activation of GLP-1’s direct incretin effect is not achieved.

Although we cannot completely exclude that the present results represent a phenomenon particular to canine incretin physiology, we believe that the present findings are concordant with the results of others (15, 28) in healthy humans. During a meal, infusion of systemic GLP-1 that resulted in peripheral GLP-1 plasma levels similar to ours did not produce a higher insulin response than the saline control. On the other hand, the significance of these human studies is unclear because better glucose tolerance during a meal could have been due to impaired gastric emptying, a known effect of the peptide (28). That possible confounder was avoided in the present studies, where glucose was given systemically, bypassing gastrointestinal absorption. Indeed, with identical glucose infusion rates in the two infusion experiments (glucose plus GLP-1 or glucose alone), very similar peripheral glucose profiles were obtained, supporting the finding that peripheral GLP-1 had little effect on the insulin response.

If systemic GLP-1 (or glucose itself) is not particularly insulinotropic under the circumstances of our experiments, what accounts for the robust insulin release seen during the meal? As seen during the infusion experiments, glucose alone produced ~53% of the meal insulin response. This is concordant with studies in humans (27) where intravenous glucose infusion isoglycemic to an oral glucose load led to a β-cell response that amounted to 41 ± 5% of the oral administration effect. In normal circumstances, other factors, such as sensory signals related to the cephalic phase of insulin secretion, contribute to the insulin secretory effect (3); due to the oral gavage administration of the meal in our study, they can be considered negligible. NEFA, which are other known stimulants of insulin release, were not significantly increased during the meal.

Glucose-dependent insulinoctropic polypeptide (GIP) released during a meal could be responsible for ~50% of the insulin secreted during a meal in humans (37). Although it was not measured in our study, it is probable that GIP increased during the meal test. GIP has a well-established insulinoctropic effect and is even considered by some researchers to be the more relevant incretin in humans, although less data comparing the incretin effect of the two peptides are available for dogs. In man, it has been shown (26, 27) that GIP can account for as much as 75% of the incretin effect. Moreover, although some
studies (27, 37) suggest that the effects of GLP-1 and GIP are additive, other results (11) indicate a synergistic effect. In this case, simulation of both meal test GLP-1 and GIP (together with glucose) via a systemic infusion might have produced the insulinotropic effect of the meal. However, the main goal of our study was not to reproduce the meal test’s insulin effect but to delineate the role of GLP-1 as an incretin, namely to determine whether addition of GLP-1 to the glucose infusion, at concentrations similar to the meal test, will produce an increase in insulin beyond that produced by glucose alone.

It is, of course, possible that GLP-1 does exert an important incretin effect, but that the effect is mediated not by systemic but by the portal concentration of the peptide secreted by the L cells of the gut. We and others (2, 4) have suggested that, but by the portal concentration of the peptide secreted by the L cells of the gut. We and others (2, 4) have suggested that, but by the portal concentration of the peptide secreted by the L

In conclusion, our study suggests that, in dogs with postprandial rises in peripheral glucose and higher than postprandial rises in GLP-1, the hormone might not have a direct insulinotropic effect. It is possible that, in such circumstances, GLP-1 exerts its effects on regulating glycemia via portal-hepatic-initiated indirect mechanisms, as well as via inhibition of glucagon, delaying gastric emptying and other insulin-independent mechanisms.

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