Intraduodenal milk protein concentrate augments the glycemic and food intake suppressive effects of DPP-IV inhibition

Diana R. Olivos, Lauren E. McGrath, Christopher A. Turner, Orianne Montaubin, Elizabeth G. Mietlicki-Baase, and Matthew R. Hayes

1Translational Neuroscience Program, Department of Psychiatry, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania

Submitted 26 July 2013; accepted in final form 13 December 2013

Intraduodenal milk protein concentrate augments the glycemic and food intake suppressive effects of DPP-IV inhibition. Am J Physiol Regul Integr Comp Physiol 306: R157–R163, 2014. First published December 18, 2013; doi:10.1152/ajpregu.00358.2013.—Glucagon-like peptide-1 (GLP-1) is an incretin hormone released from intestinal L-cells in response to food entering into the gastrointestinal tract. GLP-1-based pharmaceuticals improve blood glucose regulation and may hold promise for obesity treatment, as GLP-1 drugs reduce food intake and body weight in humans and animals. In an effort to improve GLP-1 pharmacotherapies, we focused our attention on macronutrients that, when present in the gastrointestinal tract, may enhance GLP-1 secretion and improve glycemic regulation and food intake suppression when combined with systemic administration of sitagliptin, a pharmacological inhibitor of DPP-IV (enzyme responsible for GLP-1 degradation). In particular, previous data suggest that specific macronutrient constituents found in dairy foods may act as potent secretagogues for GLP-1 and therefore may potentially serve as an adjunct dietary therapy in combination with sitagliptin. To directly test this hypothesis, rats received intraperitoneal injections of sitagliptin (6 mg/kg) or saline vehicle followed by intraduodenal infusions of either milk protein concentrate (MPC; 80/20% casein:whey; 4 kcal), soy protein (nonadry control infusate; 4 kcal), or 0.9% NaCl. Food intake was assessed 30 min postinfusion. In separate studies, regulation of blood glucose was examined via a 2-h oral glucose tolerance test (2 g/kg) following identical sitagliptin treatment and intraduodenal nutrient infusions. Collectively, results show that intraduodenal MPC, but not soy protein, significantly enhances both the food intake suppression and improved control of blood glucose produced by sitagliptin. These data support the hypothesis that dietary intake of dairy protein may be beneficial as an adjunct behavioral therapy to enhance the glycemic and food intake suppressive effects of GLP-1-based pharmacotherapies.

obesity; diabetes; dairy; insulin; GLP-1

Address for reprint requests and other correspondence: M. R. Hayes: 125 S. 31st St., Philadelphia, PA 19104 (e-mail: hayesmr@mail.med.upenn.edu).

http://www.ajpregu.org 0363-6119/14 Copyright © 2014 the American Physiological Society R157
MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (Charles River Laboratories; 330–420 g, 11–13 wk of age at the beginning of testing) were housed individually in hanging wire-bottom metal cages and maintained on a 12-h light/12-h dark cycle (lights on at 0800 h). All rats had ad libitum access to rodent chow (Purina 5001 Rodent Diet) and water except as noted. All procedures conformed to and received approval by the institutional standards of the University of Pennsylvania Animal Care and Use Committee.

Surgery

As nutrients emptying from the stomach into the small intestine first enter the duodenum where digestion and absorption begin, we chose this as the site to experimentally infuse proteins. Briefly, rats were implanted with intraduodenal catheters under ketamine (90 mg/kg), xylazine (2.7 mg/kg), and acepromazine (0.64 mg/kg) anesthesia and analgesia (Metacam, 2 mg/kg). Briefly, as described previously (20, 21, 26, 59), intraduodenal catheter implantation consisted of the insertion of a 21-cm silicone rubber catheter (0.025” inner diameter, 0.047” outer diameter, VWR, Bridgeport, NJ) into the duodenum 2 cm distal to the pylorus and advancement 5 cm within the duodenal lumen in an aboral direction. The exposed end of the catheter exited subcutaneously through a skin incision between scapulas and was occluded with a stainless steel obturator, which was removed only for flushing of the catheter (0.3 ml of 0.9% NaCl) every other day and for experimental infusions. A minimum of 7 days was allowed for recovery before experimental infusions.

Drugs and Infusates

The DPP-IV inhibitor sitagliptin monophosphate, NaCl, and (+)-d-glucose were obtained from Sigma Aldrich. MPC 85 was obtained from Sports Supplements (Colchester, UK). Soy protein was obtained from Genisoy. Tween 80, the emulsifier for the intraduodenal protein from Sports Supplements (Colchester, UK). Soy protein was obtained

Food Intake Studies

For all studies, rats were habituated to experimental procedures for 1 wk before testing. For all conditions, rats were deprived of food overnight (16 h). Each rat received counterbalanced intraportal (ip) injections of the DPP-IV inhibitor sitagliptin (6 mg/kg; dose chosen from Ref. 47) or vehicle (0.9% NaCl; 1 ml/kg) followed immediately by an 8-ml intraduodenal infusion lasting 20 min (0.4 ml/min; 300 mosmol; pH 7.35–7.4) using a syringe infusion pump (Harvard Apparatus, South Natick, MA). The total 8-ml infusion volume and rate is within the physiological range of gastric distension and emptying (13, 22, 32, 57). In separate experiments, infusions of emulsified MPC (0.5 kcal/ml MPC in 0.9% NaCl with 1.5% Tween 80) were counterbalanced with vehicle (0.9% NaCl with 1.5% Tween 80), and infusions of emulsified soy protein (0.5 kcal/ml soy protein in 0.9%NaCl with 1.5% Tween 80) were counterbalanced with vehicle (0.9% NaCl with 1.5% Tween 80). The caloric concentration of 4 kcal per infusion was selected because it is subthreshold for effect alone on subsequent 30-min food intake (see Fig. 1), is <5% of the average total daily caloric intake of adult male rats, and is within the range of previous reports examining feeding effects of intraintestinal nutrient infusions in rats (8, 20, 26, 37). Preweighed food was returned 5 min after infusions and subsequent chow intake was measured at 30 min with spillage accounted for via crumb papers below the cages. All conditions were separated by a minimum of 48 h.

Glycemic Studies

The glycemic effects of combined DPP-IV inhibition with intraduodenal protein infusions were analyzed via an oral glucose tolerance test. Similar to the feeding studies, rats were overnight food deprived (16 h) to ensure an empty gastrointestinal tract before testing. At the start of experimental testing, water bottles were removed from the animals’ home cages. Blood was collected from the tail tip of each rat, and blood glucose was measured by a standard glucometer (Accuchek, Roche Diagnostics) to determine baseline blood glucose concentrations (time = −20 min). Next, rats received an intraperitoneal injection of sitagliptin (6 mg/kg) or vehicle followed immediately by an intraduodenal infusion for 20 min as described for the feeding studies. Thus, in separate experiments, infusions of emulsified MPC (0.5 kcal/ml) were counterbalanced with vehicle, and separately, infusions of emulsified soy protein (0.5 kcal/ml) were counterbalanced with vehicle. Immediately after intraduodenal nutrient infusions, blood glucose concentrations were again assessed, and rats then received an oral glucose load (25%; 2 g/kg) via gavage (time = 0 min). Thus blood glucose was measured at −20, 0, 20, 40, 60, and 120 min in relation to the timing of the glucose load. Food and water were returned at the end of testing, and experimental treatments were separated by a minimum of 48 h.

Data and Statistical Analysis

All data are expressed as means ± SE. Data were analyzed by two-way repeated measures ANOVA, followed by Student-Newman-Keuls post hoc tests when main effects or interactions were significant. All statistical analyses were performed using Statistica Software. P < 0.05 was considered statistically significant.
RESULTS

**Intraduodenal Infusion of Milk Protein Concentrate Enhances the Glycemic and Food Intake Suppressive Effects of DPP-IV Inhibition**

*Food intake (n = 7).* A main effect of MPC on food intake was observed at 30 min postfood presentation [main effect of MPC, $F_{1,8} = 12.6693; P < 0.008$]. However, planned comparisons between treatment conditions revealed that food intake was only significantly suppressed by the combination of an intraduodenal infusion of MPC and sitagliptin administration (Fig. 1A; vehicle-0.9% NaCl vs. sitagliptin-MPC, $P < 0.05$). Food intake after saline-MPC or sitagliptin-0.9% NaCl administration was similar to that observed after vehicle treatments ($P > 0.05$).

*Glycemia (n = 33).* Sitagliptin alone significantly suppressed blood glucose at 20 and 40 min postglucose load [main effect of sitagliptin, at 20 min $F_{1,32} = 9.615; P < 0.004$; at 40 min: $F_{1,32} = 50.772; P < 0.0001$]. The glycemic effect of sitagliptin was enhanced by intraduodenal infusion of MPC at 40 min postglucose load (Fig. 2: $F_{1,32} = 139.206; P < 0.01$). In addition, MPC alone produced a suppression of blood glucose at 20 min postglucose load [main effect of MPC; $F(1,32) = 269.176; P < 0.001$].

**Intraduodenal Infusion of Soy Protein Does Not Enhance the Glycemic or Food Intake Suppressive Effects of DPP-IV Inhibition**

*Food intake (n = 9).* To confirm that the enhanced suppression of food intake produced by the combination of sitagliptin and MPC was specific to dairy protein and to rule out the possibilities that the effect could be broadly attributed to any intraintestinal protein or caloric load, a separate group of rats received isocaloric intraduodenal infusions of soy protein in combination with sitagliptin. The combination of soy protein infusion with sitagliptin administration had no significant effect on 30 min chow intake (Fig. 1B; $P > 0.05$).

*Glycemia (n = 24).* Similar to the feeding studies, we wanted to determine whether the improved glycemic regulation observed by MPC and sitagliptin combination was specific to dairy protein rather than any intraintestinal protein or caloric load. Thus a separate group of rats received intraduodenal infusions of isocaloric soy protein in combination with sitagliptin administration. As expected, sitagliptin alone produced a significant suppression of blood glucose at 20 and 40 min postglucose load [main effect of sitagliptin, at 20 min $F_{1,23} = 35.824; P < 0.0001$; at 40 min $F_{1,23} = 29.845; P < 0.0001$]. Soy protein alone reduced blood glucose at 20 and 40 min postglucose load [main effect of soy, at 20 min $F_{1,23} = 51.114; P < 0.0001$; at 40 min $F_{1,23} = 46.393; P < 0.0001$]. However, unlike the enhanced suppression of blood glucose observed with MPC, infusion of soy protein did not produce a significant enhancement in the glycemic suppressive effects of sitagliptin at any time point (Fig. 3; $P > 0.05$). Figure 4 shows the percent suppression of blood glucose concentrations following intraduodenal infusion of soy protein compared with MPC with and without sitagliptin administration. Between-subject analyses of this percent suppression shows that the combination of MPC with sitagliptin produced a significantly increased percent suppression in blood glucose compared with soy protein and sitagliptin administration at 40 min ($P < 0.05$).

DISCUSSION

Both DPP-IV inhibitors and GLP-1R agonists are FDA approved to treat T2DM by improving blood glucose regulation, but importantly, neither class of drug is a “cure” for T2DM. Given that with any pharmaceutical there is always the risk of tachyphylaxis (reduced response to a drug due to previous long-term exposure to that drug), identifying complementary and alternative/homeopathic therapies to GLP-1-
based pharmaceuticals is a necessary step in the long-term control and treatment of T2DM. As accumulating data support the notion that regular consumption of dairy foods may have a beneficial role in the prevention of the metabolic syndrome (1, 11, 34, 35, 49, 60, 61), the rationale for identifying the specific dairy constituent(s) and mechanism of action for the putative beneficial effects of dairy products on glycemic regulation is clear. As dairy-enriched whole proteins and amino acids can elicit GLP-1 secretions in vitro (7, 15), as well as inhibit intestinal DPP-IV activity in vivo (17), we sought to determine whether the glycemic and feeding effects produced by acute pharmacological inhibition of DPP-IV by sitagliptin could be enhanced when combined with intraduodenal MPC infusion. Current findings show that intraduodenal infusion of MPC, but not soy protein, significantly enhances both the short-term food intake and glycemic suppressive effects of sitagliptin. These data support the hypothesis that dietary intake of dairy protein may be beneficial as an adjunct behavioral therapy to enhance the glycemic and food intake suppressive effects of select GLP-1-based pharmacotherapies.

The amount of endogenous postprandial GLP-1 released from intestinal L-cells in humans is correlated with the size and macronutrient composition of the meal (4, 43). This is an important concept when considering not only the dietary recommendations that should accompany prescribed DPP-IV inhibition for T2DM treatment, but also the relevant GLP-1R population(s) within the periphery that may respond to secreted GLP-1 to control for blood glucose utilization. Indeed, within the periphery there are distinct populations of GLP-1Rs relevant to glycemic control, including GLP-1R expressed on pancreatic β-cells, as well as vagal afferent fibers innervating the GI tract and/or hepatoportal bed (see Refs. 23 and 25 for review). Under normal physiological conditions, GLP-1 paracrine-like signaling and activation of vagal afferent fibers innervating the intestine appears to be the predominant mode of action controlling for intestinally derived GLP-1 glycemic effects (23–25, 54). An important limitation to the current findings is the absence of plasma GLP-1 and insulin measures. Nonetheless, with the presumed elevation in endogenous GLP-1 signaling produced by pharmacological DPP-IV inhi-
bition in the present studies, it is possible that any of the aforementioned GLP-1R populations could mediate the enhanced suppression of blood glucose concentration by the combination of MPC with sitagliptin.

The strength of the peripheral GLP-1 system as a candidate for obesity treatment is highlighted by previous reports showing that endogenous peripherally secreted GLP-1 is physiologically important not only for glycemic control (24, 58) but also for the control of food intake (55). For example, systemic administration of the GLP-1R antagonist exendin-(9–39) in rats has been reported to impair glucose tolerance (24) and attenuate the intake-suppressive effects that follow voluntary consumption and intragastric infusion of a liquid meal (55). However, other reports indicate an absence of hyperphagia following systemic GLP-1R antagonist administration (41). While the discrepancies in the aforementioned antagonist-driven feeding effects may be a consequence of paradigmatic differences, the sustained glycemic- and food intake-suppressive effects that can be achieved by targeting the GLP-1 system with daily administration of DPP-IV inhibitors or GLP-1R agonists are clear (see Refs. 19, 29, 31, and 44 for review). Although less potent in their physiological and behavioral effects than the GLP-1R agonists, DPP-IV inhibitors offer humans the advantage of reduced incidence of adverse effects (e.g., nausea/vomiting) and the opportunity for an oral route of administration (10, 29). Interestingly, a previous report by Reimer et al. (38) showed that chronic elevation of endogenous GLP-1 signaling by DPP-IV inhibition produces a sustained suppression in food intake. Specifically, these authors reported that mice treated with the DPP-IV inhibitor NVP DPP728 in their drinking water had reduced weekly food intake when maintained on either standard rodent chow or high-fat diet (38). Together with the current acute feeding data, it seems logical to investigate whether the sustained suppression of weekly food intake observed in the aforementioned report could be further enhanced by the maintenance of animals on a diet high in MPC. Moreover, as DPP-IV inhibition does not selectively affect GLP-1 signaling but also can delay the degradation rate of other neuropeptides (e.g., gastric inhibitory polypeptide, vasoactive intestinal peptide, and substance P) (48), future research is also warranted to investigate potential alternative non-GLP-1-mediated pathways for the observed effects.

Perspectives and Significance

The collective set of data suggests that the presence of MPC within the small intestine can augment endogenous GLP-1 signaling, an effect important for both food intake and glycemic control, as the combination of acute DPP-IV inhibition together with an intraduodenal infusion of MPC resulted in a significant enhancement in the suppression of food intake and blood glucose concentrations. This hypothesis is also supported by clinical (5, 9, 18, 30, 42, 43) and basic science (45, 62) reports showing that ingestion or intraintestinal infusion of dairy proteins, oligopeptides, and isolated amino acids can elevate postprandial plasma GLP-1 and other intestinally derived gut peptide (e.g., cholecystokinin) concentrations. What remains to be determined is whether the putative increase in GLP-1 signaling following intraintestinal exposure to MPC is a consequence of increased GLP-1 secretions by L-cells and/or a consequence of reduced bioactive DPP-IV levels. Support for both possibilities is provided by evidence demonstrating that several oligopeptides can act as endogenous inhibitors of DPP-IV (17, 33), as well as a report by Gunnarsson et al. (17) showing that whey protein administration in mice reduces DPP-IV activity in the proximal small intestine, the predominant site of GLP-1 secretion in the intestine. Additionally, in vitro reports have shown that when GLP-1-producing L-cells are exposed to whole dairy proteins or specific dairy-enriched amino acids (i.e., leucine and isoleucine), there is an increase in GLP-1 secretion (7, 15). Thus as MPC is such a complex and diverse source of amino acids, oligopeptides, and complete proteins (i.e., casein and whey), it is not clear if the current results would be recapitulated with an isolated infusion of one or more of these dairy-based protein constituents. However, the absence of enhanced effects observed when sitagliptin was combined with intraduodenal infusion of soy protein suggests that the feeding and glycemic responses observed for MPC are not simply the consequence of intraduodenal protein in general or by the total caloric infusion, but rather represent some specific aspect of MPC. Thus further studies are certainly warranted to elucidate not only the physiological mechanisms by which dairy protein can enhance the glycemic and feeding effects of pharmacological DPP-IV inhibition, but also to investigate whether these effects would be recapitulated in hyperglycemic animal models and T2DM/prediabetic humans when treated daily with a DPP-IV inhibitor and maintained on a high dairy protein-based diet.

ACKNOWLEDGMENTS

Valuable technical assistance was provided by Derek Zimmer, Brianne Jeffery, and Sky Prestowitz.

GRANTS

This research was supported by National Institutes of Health Grants NIH-DK085435 and DK096139, as well as an investigator-initiated research grant from “Dairy Management Inc.” and administered by the Dairy Research Institute (no. 1067).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES

DAIRY PROTEIN ENHANCES METABOLIC EFFECTS OF DPP-4 INHIBITION

10. Flegal KM, Carroll MD, Ogden CL, Curtin LR.
17. Fraser KA, Raizada E, Davison JS.
19. Hayes MR, Bradley L, Grill HJ.
21. Holst JJ.
22. Hayes MR, De Jonghe BC, Kanoski SE.
23. Hayes MR, Chory FM, Gallagher CA, Covasa M.
24. Gunnarsson PT, Winzell MS, Deacon CF, Larsen MO, Jelic K, Carr Giralt M, Vergara P.
25. Geraedts MC, Troost FJ, Fischer MA, Edens L, Saris WH.
26. Diepvens K, Haberer D, Westerterp-Plantenga M.