0013-7227/06/$15.00/0
Printed in U.S.A.

Endocrinology 147(7):3171–3172

doi: 10.1210/en.2006-0494

Enhancing the Action of Incretin Hormones: A New Whey Forward?

The gastrointestinal tract is an often overlooked but complex endocrine organ, home to dozens of regulatory peptides produced in specialized endocrine cells and enteric neurons. These hormones subserve complex roles as signals regulating appetite, gastrointestinal motility, control of secretion from the exocrine and endocrine pancreas, and nutrient absorption (1). The majority of gut peptides are secreted within minutes of nutrient ingestion and rise transiently in the circulation, with levels rapidly falling back to basal levels after termination of feeding. Because complex disorders such as obesity and diabetes involve imbalances in the control of energy ingestion and disposal, there is considerable interest in understanding the physiological role and therapeutic potential of gut peptides in the control of nutrient assimilation.

Two enteroendocrine-derived peptides, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), play important roles in preparing the pancreas to handle an incoming nutrient load. Both GIP and GLP-1 function as incretin hormones, gut-derived peptides that potentiate insulin secretion from the islet β-cell in a glucose-dependent manner (2, 3). Considerable recent evidence suggests that incretin-based therapies may be useful for the treatment of type 2 diabetes because continuous administration of GLP-1 produces substantial improvements in glucose control and β-cell function in subjects with type 2 diabetes (4). However, the rapid degradation of both GIP and GLP-1 by the aminopeptidase, dipeptidyl peptidase-4 (DPP-4), has fostered the development of degradation-resistant GLP-1R agonists such as exendin-4 (Exenatide), now employed as a twice daily injectable agent for the treatment of type 2 diabetes (5). Complementary efforts to prolong incretin action include the use of chemical inhibitors of DPP-4 activity, and several DPP-4 inhibitors have completed extensive clinical testing in subjects with type 2 diabetes.

In the current issue of *Endocrinology*, Gunnarsson (6) *et al*. illuminate an additional approach for enhancing incretin action via administration of nutrients with selective effects on potentiation of incretin secretion and incretin degradation. Both oleic acid and whey protein (WP) enhanced the insulin response to glucose in mice; however, only whey protein was associated with an increase in the levels of intact bioactive incretin hormones. Although oleic acid and whey protein significantly increased the levels of circulating intact GLP-1 after glucose administration, the ratio of intact:total GLP-1 was similar in mice receiving glucose alone *vs.* glucose plus oleic acid or whey protein. In contrast, WP administration did not increase the levels of total circulating GIP but did significantly increase the levels of intact GIP. The authors then investigated whether the changes in levels of circulating incretin hormones were associated with differences in DPP-4 activity in the plasma or gastrointestinal tract. Remarkably, whey protein administration produced a significant reduction in DPP-4 activity in the proximal small bowel, the predominant site of GIP synthesis, whereas no changes in DPP-4 activity were observed in the distal gut or plasma. The authors speculate that the reduction in DPP-4 activity in the proximal small bowel may directly account for the preferential increase in levels of intact GIP detected after glucose and whey protein challenge in their studies. Although the mechanism(s) underlying these associations remain unclear, one potential explanation offered is the generation of protein fragments (dipeptides or tripeptides) after digestion of whey protein that may serve as endogenous inhibitors of DPP-4 activity in the proximal gut.

What are the implications of this study for efforts directed at prolonging incretin action for the treatment of type 2 diabetes? Nutrients are potent stimulators of incretin secretion, and nutraceuticals such as oligofructose may exert beneficial effects in the control of body weight and glucose disposal in part by enhancing the secretion of GLP-1 from the distal gut (7). Much less is known about the potential for various nutrients to differentially regulate incretin degradation. Both GIP and GLP-1 contain an alanine at position 2, and DPP-4 is the principal enzyme responsible for the rapid degradation of these peptides *in vivo*. DPP-4 is essential for inactivation of both GLP-1 and GIP because mice with a targeted inactivation of the DPP-4 gene exhibit reduced glycemic excursion after glucose challenge, in association with increased circulating levels of insulin and intact GLP-1 and GIP. DPP-4 is a complex molecule that exists as a cell surface membrane-spanning enzyme widely expressed on numerous cell types, and as a soluble circulating form abundant in the circulation. Current pharmaceutical efforts targeting DPP-4 inhibit the enzymatic activity of both the membrane-associated and soluble forms of the enzyme wherever they may be found, in multiple tissue compartments or in the circulation.

The current studies suggest that selective inhibition of DPP-4 in the proximal gut may produce detectable increases in levels of intact incretin hormones, as demonstrated in the present instance for GIP. Several oligopeptides have been described as inhibitors of DPP-4, including the N-terminal nine amino acids of the HIV-1 Tat protein, which is capable of binding to the active site of DPP-4 and inhibiting DPP-4 enzymatic activity (8). Similarly, the opioid dynorphin-A (1–17) peptide has been shown to down-regulate DPP-4 activity on the cell surface of R1.1 cells, an immature T lymphocyte cell line (9). Because DPP-4 is abundant in the gas-

Abbreviations: DPP-4, Dipeptidyl peptidase-4; , GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

trointestinal tract and is expressed on endothelial cells directly adjacent to incretin-secreting enteroendocrine cells (10), inhibition of intestinal DPP-4 may be a useful strategy for enhancing incretin action for the treatment of diabetes. The importance of intestinal DPP-4 for control of incretin degradation is illustrated by observations demonstrating that the majority of circulating incretin hormones represent the cleaved inactive moieties (11), implying very rapid degradation of both GLP-1 and GIP very soon after their release from enteroendocrine cells. The ability of Gunnarson and colleagues to detect significantly increased levels of intact GIP (1–42) in the plasma of mice after whey protein administration implies even higher levels of intact GIP traversed the portal system and may account in part for the enhanced insulin response to glucose administration.

What are the implications of these findings for scientists and clinicians interested in the control of incretin degradation and DPP-4 activity? The current studies employed acute administration of whey protein and glucose to mice, a situation not necessarily relevant to complex nutrient ingestion in normal or diabetic human subjects. Whether chronic administration of whey protein will produce sustained inhibition of intestinal DPP-4 activity in normal or diabetic animals merits further study. Rats fed a high whey protein diet for 6 wk exhibited improvements in insulin sensitivity and reduced weight gain; however, levels of incretin hormones were not reported in this study (12). Most intriguingly, acute administration of whey protein-supplemented meals to human subjects with type 2 diabetes was associated with significant increments in postprandial insulin responses and higher levels of GIP but not GLP-1 (13), consistent with the data reported in the current murine study by Gunnarsson *et al.* That whey protein preferentially stimulates an increase in levels of intact GIP, classically produced in duodenal and jejunal K cells, but not levels of intact GLP-1, a hormone long thought to be secreted by the distal gut, provides further insight into the relative importance of proximal *vs.* distal L cells as sources of GLP-1 secretion. Recent studies have implied that the rapid increase in GLP-1 secretion observed after meal ingestion may be attributable to GLP-1-producing L cells in the proximal small bowel (14, 15) rather than signals arising from the proximal gut that rapidly stimulate GLP-1 secretion from distal L cells (16). The current observations call into question the quantitative importance of GLP-1 produced from and secreted by duodenal or jejunal enteroendocrine cells because whey protein failed to produce significant increases in the ratio of intact:total GLP-1 despite marked inhibition of DPP-4 activity in the proximal small bowel. Taken together, the studies by Gunnarsson *et al.* remind us how little we know about the factors regulating the expression and activity of DPP-4 in different tissue compartments. Given the current enthusiasm for inhibition of DPP-4 activity as a potential new therapy for the treatment of type 2 diabetes, a greater understanding of the biology of DPP-4 synthesis, activation, and clearance, seems timely.

Daniel J. Drucker
Department of Medicine and the Banting and Best Diabetes Centre
Toronto General Hospital
University of Toronto
Toronto, Ontario, Canada M5G 2C4

Acknowledgments

Received April 17, 2006. Accepted April 18, 2006.

Address all correspondence and requests for reprints to: Dr. D. Drucker, Toronto General Hospital, 200 Elizabeth Street MBRW4R-402, Toronto Ontario, Canada M5G 2C4.

D.J.D. is supported by a Canada Research Chair in Regulatory Peptides.

References

1. **Badman MK, Flier JS** 2005 The gut and energy balance: visceral allies in the obesity wars. Science 307:1909–1914
2. **Creutzfeldt W, Ebert R** 1985 New developments in the incretin concept today. Diabetologia 28:565–573
3. **Drucker DJ** 2006 The biology of incretin hormones. Cell Metab 3:153–165
4. **Zander M, Madsbad S, Madsen JL, Holst JJ** 2002 Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and β-cell function in type 2 diabetes: a parallel-group study. Lancet 359:824–830
5. **Riddle MC, Drucker DJ** 2006 Emerging therapies mimicking the effects of amylin and glucagon-like peptide 1. Diabetes Care 29:435–449
6. **Gunnarsson PT, Winzell MS, Deacon CF, Larsen MO, Jelic K, Carr RD, Ahrén B** 2006 Glucose-induced incretin hormone release and inactivation are differently modulated by oral fat and protein in mice. Endocrinology 147: 3173–3180
7. **Cani PD, Knauf C, Iglesias MA, Drucker DJ, Delzenne NM, Burcelin R** 2006 Improvement of glucose tolerance and hepatic insulin sensitivity by oligofructose requires a functional glucagon-like peptide 1 receptor. Diabetes 55: 1484–1490
8. **Mrestani-Klaus C, Fengler A, Faust J, Brandt W, Wrenger S, Reinhold D, Ansorge S, Neubert K** 2000 N-terminal HIV-1 Tat nonapeptides as inhibitors of dipeptidyl peptidase IV. Conformational characterization. Adv Exp Med Biol 477:125–129
9. **Gabrilovac J, Abramic M, Uzarevic B, Andreis A, Poljak L** 2003 Dipeptidyl peptidase IV (DPPIV) enzyme activity on immature T-cell line R1.1 is down-regulated by dynorphin-A(1–17) as a non-substrate inhibitor. Life Sci 73:151–166
10. **Hansen L, Deacon CF, Orskov C, Holst JJ** 1999 Glucagon-like peptide-1-(7–36)amide is transformed to glucagon-like peptide-1-(9–36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. Endocrinology 140:5356–5363
11. **Deacon CF, Johnsen AH, Holst JJ** 1995 Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite *in vivo*. J Clin Endocrinol Metab 80:952–957
12. **Belobrajdic DP, McIntosh GH, Owens JA** 2004 A high-whey-protein diet reduces body weight gain and alters insulin sensitivity relative to red meat in Wistar rats. J Nutr 134:1454–1458
13. **Frid AH, Nilsson M, Holst JJ, Bjorck IM** 2005 Effect of whey on blood glucose and insulin responses to composite breakfast and lunch meals in type 2 diabetic subjects. Am J Clin Nutr 82:69–75
14. **Mortensen K, Christensen LL, Holst JJ, Orskov C** 2003 GLP-1 and GIP are colocalized in a subset of endocrine cells in the small intestine. Regul Pept 114:189–196
15. **Theodorakis MJ, Carlson O, Michopoulos S, Doyle ME, Juhaszova M, Petraki K, Egan JM** 2006 Human duodenal enteroendocrine cells: source of both incretin peptides, GLP-1 and GIP. Am J Physiol Endocrinol Metab 290: E550–E559
16. **Brubaker PL, Anini Y** 2003 Direct and indirect mechanisms regulating secretion of glucagon-like peptide-1 and glucagon-like peptide-2. Can J Physiol Pharmacol 81:1005–1012

Endocrinology **is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**