Physiological and Pharmacological Mechanisms through which the DPP-4 Inhibitor Sitagliptin Regulates Glycemia in Mice

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Inhibition of dipeptidyl peptidase-4 (DPP-4) activity improves glucose homeostasis through a mode of action related to the stabilization of the active forms of DPP-4-sensitive hormones such as the incretins that enhance glucose-induced insulin secretion. However, the DPP-4 enzyme is highly expressed on the surface of intestinal epithelial cells; hence, the role of intestinal vs. systemic DPP-4 remains unclear. To analyze mechanisms through which the DPP-4 inhibitor sitagliptin regulates glycemia in mice, we administered low oral doses of the DPP-4 inhibitor sitagliptin that selectively reduced DPP-4 activity in the intestine. Glp1r−/− and Gipr−/− mice were studied and glucagon-like peptide (GLP)-1 receptor (GLP-1R) signaling was blocked by an iv infusion of the corresponding receptor antagonist exendin (9–39). The role of the dipeptides His-Ala and Tyr-Ala as DPP-4-generated GLP-1 and glucose-dependent insulinotropic peptide (GIP) degradation products was studied in vivo and in vitro on isolated islets. We demonstrate that very low doses of oral sitagliptin improve glucose tolerance and plasma insulin levels with selective reduction of intestinal but not systemic DPP-4 activity. The glucoregulatory action of sitagliptin was associated with increased vagus nerve activity and was diminished in wild-type mice treated with the GLP-1R antagonist exendin (9–39) and in Glp1r−/− and Gipr−/− mice. Furthermore, the dipeptides liberated from GLP-1 (His-Ala) and GIP (Tyr-Ala) deteriorated glucose tolerance, reduced insulin, and increased portal glucagon levels. The predominant mechanism through which DPP-4 inhibitors regulate glycemia involves local inhibition of intestinal DPP-4 activity, activation of incretin receptors, reduced liberation of bioactive dipeptides, and activation of the gut-to-pancreas neural axis. (Endocrinology 152: 0000–0000, 2011)

During a meal, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are secreted into the mesenteric and hepatoportal veins (1, 2). Upon binding to their respective receptors on insulin-secreting β-cells, both peptides enhance glucose-dependent insulin secretion and improve glycemic control (3, 4). This mechanism underlies the incretin effect (5). Hence, both peptides have drawn interest for development of innova-
tive therapeutic strategies based on enhancing incretin action. However, one major drawback to the use of native GLP-1 and GIP is that the peptides are rapidly degraded by an endoprotease, dipeptidyl peptidase-4 (DPP-4) as soon as they are secreted by enteroendocrine L and K cells, respectively (6, 7). Dpp4−/− mice exhibit reduced glycemic excursion after a glucose challenge in association with increased levels of glucose-stimulated insulin and the intact insulinotropic forms of GLP-1 and GIP (8), illustrating the importance of endogenous DPP-4 for control of the incretin axis.

Two new therapeutic strategies are now available based on potentiation of incretin action, one employing injectable DPP-4-resistant GLP-1 receptor (GLP-1R) agonists and a second approach involving orally active inhibitors of DPP-4. The development of DPP-4-resistant GIP analogs has also been initiated for the treatment of type 2 diabetes (9, 10), and clinical trials are ongoing to validate their efficacy (11). The administration of either liraglutide or exenatide, the approved GLP-1R agonists, results in pharmacological levels of these peptides, increases in plasma insulin, decreases in glucagon, and improvement in glycaemic control (12–14). In contrast, DPP-4 inhibitors prevent degradation of endogenous incretin hormones, stabilizing intact concentrations of GLP-1 and GIP in the mesenteric and hepatopancreatic veins, close to the site of intestinal incretin secretion. However, the increase in circulating intact GLP-1 levels is often modest, and whether this small increase in plasma GLP-1 engages the β-cell GLP-1R, accounting for the improvement in glycemic control observed after DPP-4 inhibition, is unclear. Therefore, whether endogenous GLP-1 acts as a true circulating incretin hormone in the context of DPP-4 inhibition remains to be determined (15).

We and others previously showed that in the awake free-moving mouse and other animal models, a vagal hepatopancreatic reflex (16) is initiated by activation of the hepatoportal glucose sensor to control peripheral glucose utilization in a GLP-1R-dependent manner (17–21). This mechanism required the simultaneous activation of brain GLP-1 signaling to trigger control of glucose-regulated insulin secretion (22), muscle blood flow and insulin sensitivity (23), and hepatic glucose production and food intake (24, 25). Together, these data show that a physiological increase in GLP-1 regulates glucose metabolism through a mechanism that involves the enteropancreatic gut-to-brain-to-periphery axis (26).

These findings raise the possibility that the glucoregulatory actions of DPP-4 may involve local regulation of the GLP-1-dependent gut-to-brain-to-periphery axis. To test this hypothesis, we inhibited DPP-4 activity in the intestine using a very low oral dose of sitagliptin and show that selective local reduction in intestinal DPP-4 activity is sufficient for activation of the neurally mediated gut-to-brain-to-periphery axis. Moreover, reduced liberation of the bioactive dipeptides released from GLP-1 (His-Ala) and GIP (Tyr-Ala) may also contribute to the therapeutic effects of DPP-4 inhibition.

**Materials and Methods**

**Animals and research design**

Eleven-week-old C57BL/6j (Charles River, L’Arbresle, France), Gipr−/− and Glpr−/− male mice were housed in a controlled environment (inverted 12-h daylight cycle, lights off at 1000 h) with free access to food and water. All the following animal experimental procedures have been approved by the local ethical committee of the Rangueil hospital or the Mt. Sinai Hospital/Toronto Center for Phenogenomics. Mice were fed a normal carbohydrate diet (energy content of 12% fat, 28% protein, and 60% carbohydrate; A04, Safe, France). Intrafemoral and intraportal catheters were indwelled for glucose and drug administration. A few days after recovery from surgery, the catheters were connected to infusion systems, allowing the animal to remain in its cage. This allows for injections without handling the animals. Mice underwent a glucose tolerance test as described below. Mice allocated to the recording of vagus nerve activity were infused with the GLP-1R antagonist exendin (9–39) (Ex9) into the femoral catheter (0.1 nmol/kg/min) for 45 min before the intragastric glucose challenge and until the end of the experimental procedure. We defined this small dose in previous experiments to be fully functional in the mouse (17). In all the other sets of mice, a higher dose of Ex9 was flash injected (1 nmol/mouse) to pharmacologically inhibit GLP-1R signaling. We used this high dose to ensure a full inhibition of the GLP-1R because the ip administration might not allow homogeneous distribution of the antagonist peptide into the body.

**Surgical procedures**

For intraportal or femoral catheters, the mice were anesthetized with isoflurane (Abbott, Rungis, France). A catheter was indwelled into the femoral vein (23) or the portal vein (17, 27, 28). After insertion of catheters, mice were allowed to recover for a full week to reach their presurgical body weight. Mice that did not recover completely were not used in additional experiments.

**Glucose tolerance test**

In a first set of experiments, a glucose solution (30%) was administered (2 g/kg) orally or iv in mice fasted for 6 h. This high-glucose dose still resulted in rapid normalization of glycemia when administered iv. The mice were gavaged with the DPP-4 inhibitor sitagliptin (Merck Sharp Dohme and Chibret Laboratories, Rahway, NJ) diluted in 200 μl water at different concentrations ranging from 4–400 μg/mouse 30 min before the glucose challenge. In a few instances, we used the dose of 40 mg/mouse to totally inhibit intestinal and systemic DPP-4 activity. No physiological conclusion could be drawn using 40 mg sitagliptin because this dose completely inhibited gastric emptying.

Another set of mice was allocated for assessment of portal vein GLP-1 and GIP concentrations. Sitagliptin was also admin-
istered 30 min before the glucose challenge. Then, after the glucose challenge, blood was sampled in the presence of diprotin A (ile-pro-ile, 0.1 mmol/liter; Sigma-Aldrich, St. Louis, MO) and heparin 15 min after the glucose challenge, i.e. 45 min after the administration of sitagliptin, as follows. A rapid anesthesia (less than 5 min) was induced by an ip injection of a mix of ketamine 1000 (Virbac, Carros, France) xylazine (rompum 2%, 100 and 10 mg/kg ip, respectively; Bayer HealthCare, Loos, France) to perform the portal vein blood samplings. Because 250 µl portal blood was required and collected at each time point, one set of mice was allocated to each time point. This procedure allows the sampling of blood before the glucose rises above 6.5 mm.

Plasma parameters

Plasma insulin concentration was determined from 10 µl using the mouse ultrasensitive insulin ELISA kit (Mercodia, Uppsala, Sweden). Plasma GLP-1 concentration was determined from 100 µl using the GLP-1 (Active) ELISA kit (Linco Research, St. Charles, MO). Plasma glucagon was determined using the RIA kit for glucagon (Millipore, Billerica, MA). Active GIP concentrations were measured as described previously (29).

Measurement of parasympathetic nervous system activity

The mice were gavaged with sitagliptin or water 30 min before the intragastric glucose challenge. Afterward, the mice were anesthetized, and vagus nerve activity was recorded at the level of the trachea as previously described (23, 30). Briefly, while the mice were kept on a heating blanket at 37 C in a Faraday cage set up, a first electrode was hooked to the vagus nerve while a second one was implanted under the skin as a reference. The vagus nerve activity was recorded using a data acquisition system (Powerlab 8/3, ADInstruments, Colorado Springs, CO). The signal was filtered between 0.1 and 1000 Hz, with a 4 koczet/sec sampling rate, and amplified by the BioAmp. After the glucose challenge, vagus nerve activity was recorded for 30 min. At completion of the recording period, 600 µg acetylcholine was injected into the peritoneal cavity. This dose was extremely high and employed to nonspecifically activate the overall system and make sure that we could obtain a response. Consequently, this dramatically increased vagus nerve activity, facilitating calibration of the recording and validating the sensitivity of the experimental set-up. Mice in which the firing rate activity of the vagus nerve was not increased by acetylcholine were not included in the analysis.

Insulin and glucagon secretion from isolated human and mouse islets

Murine islets

Mice were killed by cervical dislocation, the pancreas removed, and islets isolated by collagenase digestion and subsequently separated from the remaining exocrine tissue by hand-picking under a stereomicroscope, as previously described (31). Islets were then cultured overnight in CMRL-1066 (Invitrogen, Carlsbad, CA) supplemented with 0.25% human serum albumin, 20 mM L-glutamine, 64 mg/liter gentamicin, and 2.5 mM HEPES at 37 C in 95% O2/5% CO2 and saturated humidity in 96-well filter plates (multiscreen Durapore BV1.2 µm; Millipore) (five islets per well). In vitro insulin release was assayed under static incubation. The culture medium was replaced by Krebs-Ringer-bicarbonate-HEPES buffer/0.05% fatty acid-free BSA containing 5.5 or 16.7 mM glucose with or without histidine-alanine (His-Ala) dipeptides at 1, 10, and 100 mM, and islets were further incubated for 90 min at 37 C. The plates were then centrifuged, and the supernatants were stored at −20 C until assayed for insulin and glucagon.

Human islets

Human islets were isolated from 20- to 52-yr-old nondiabetic adult donors (19 kg/m2 < body mass index < 25 kg/m2) with brain death as previously described (31). The islets were hand-picked and cultured overnight in CMRL-1066 (Invitrogen). In vitro insulin release was assayed under static incubation using the same protocol followed for mouse islets.

DPP-4 assay

DPP-4 activity was assessed in the plasma or the intestinal lumen, mucosa, or the epithelium from the duodenum, jejunum, and ileum 15 min after the glucose challenge, i.e. 45 min after the administration of sitagliptin. To assess DPP-4 activity, 50 µl plasma or 30 mg of the intestinal extracts were used. Luminal contents were collected by immediate flushing of the intestine; the mucosa was hydrated with 1 ml PBS (140 mM NaCl, 3 mM KCl, 4 mM Na2HPO4, 1.5 mM KH2PO4) and collected by gentle scraping. The epithelial cells are then spun down by centrifugation (8000 rpm at 4 C for 5 min), and the supernatant was stored. We hence assessed the non-membrane-associated and the membrane-associated fractions for DPP-4 activity. Eventually, the remaining intestinal epithelium corresponding to the basal membrane of the intestine was stored in the same buffer. DPP-4 activity was assayed from 50 µl of the extracts incubated with kit reagents for 2 h at 37 C according to the manufacturers’ recommendations using recombinant DPP-4 as a standard, expressed in nanograms per milliliter (DPP-4 Glo protease assay; Promega, Madison, WI). The data are expressed as percentage of control, which corresponds to the value obtained in the absence of sitagliptin.

Statistical analyses

Results are presented as means ± SEM. Statistical analyses were performed using Prism GraphPad version 5.01 (GraphPad Software Inc., San Diego, CA). Between-group differences were analyzed using the Student’s t test for single comparisons and two-way ANOVA with Bonferroni post hoc test for multiple comparisons, and a statistical difference was considered significant when P < 0.05.

Results

Inhibition of intestinal DPP-4 by low-dose oral sitagliptin improves glucose tolerance

We first determined the minimal oral dose of sitagliptin sufficient to reduce glycemic profiles after an oral glucose challenge. Oral administration of as low as 4 µg sitagliptin per mouse improves glucose tolerance in normal healthy mice (Fig. 1, A and C). Larger doses of sitagliptin (40, 80, 120, and 400 µg) produced more robust glucose regulation (Fig. 1, B and C). Interestingly, a pharmacological dose of 40 mg almost totally blunted glycemic excursion (not shown).
due to complete inhibition of gastric emptying. Plasma insulin concentrations in the portal vein assessed 15 min after the oral glucose challenge were significantly increased by a dose of sitagliptin as low as 40 μg/mouse (Fig. 1D).

The hepatoportal concentrations of active GLP-1 in the presence of sitagliptin at doses ranging from 4–40 μg/mouse remained almost undetectable, close to the detection limit of 2 pM GLP-1. They were significantly higher after administration of 400 μg/mouse (Fig. 1E), which was also associated with a robust reduction of plasma DPP-4 activity (not shown). In contrast, active hepatoportal GIP concentrations were significantly increased with 40 and 400 μg/mouse sitagliptin (Fig. 1F). Although lower sitagliptin doses (40–120 μg) reduced glucose (Fig. 1A) and increased portal insulin levels (Fig. 1D), these bioactive doses had no effect on plasma DPP-4 activity (Fig. 1, G and I). In contrast, the maximal sitagliptin dose employed (40 mg/mouse) markedly reduced plasma DPP-4 activity and inhibited gastric emptying (Fig. 1, G and I, and data not shown). We next quantified DPP-4 activity in different compartments of the intestine, specifically the lumen, mucosa, and submucosal epithelium. The 40-μg sitagliptin dose was sufficient to reduce DPP-4 activity in multiple compartments of the duodenum and jejunum and partly in the ileum (Fig. 1, H and J).

**Low-dose parenteral sitagliptin and parameters of glucose tolerance**

The iv administration of enterally active doses of sitagliptin had only modest effects on glycemic control (Fig. 2, A and B). It is noteworthy we did not observe a similar dose-response relationship with low doses of sitagliptin given iv, relative to the effect observed when the drug was given orally (compare with Fig. 1, A and B). Portal vein insulin levels were increased by oral glucose alone but not further augmented when the low doses of sitagliptin were administered iv (Fig. 2B, inset). In contrast to the significant reduction of gut DPP-4 activity with low-dose enteral sitagliptin administration, plasma DPP-4 activity was unchanged after iv sitagliptin treatment at lower doses (4–
120 μg, see Supplemental Fig. 1, published on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org), with a significant 30% reduction observed only at 400 μg sitagliptin (Supplemental Fig. 1). Consistent with the importance of suppressing DPP-4 activity within intestinal tissue, portal vein administration of sitagliptin (40 μg/mouse) was not sufficient to change glycemic profiles, portal GLP-1, or plasma DPP-4 activity (Fig. 2, C–E). Portal GIP concentrations remained similarly unchanged (87 ± 15 vs. 92 ± 22 ng/ml in the absence or presence of 40 μg/mouse sitagliptin, respectively).

The control of glucose tolerance by intestinal DPP-4 requires GLP-1 and GIP receptors

To determine whether the GLP-1R mediated control of oral glucose tolerance by low-dose sitagliptin, we coadministered the GLP-1R antagonist Ex9 into the systemic circulation simultaneous to the oral administration of sitagliptin (40 μg/mouse) or plasma DPP-4 activity (Fig. 2, C–E). Portal GIP concentrations remained similarly unchanged (87 ± 15 vs. 92 ± 22 ng/ml in the absence or presence of 40 μg/mouse sitagliptin, respectively).

DPP-4 inhibition regulates vagus nerve activity

Enteric glucose activates the gut-to-brain axis by a mechanism that requires the GLP-1R (18, 22, 32) and the autonomic nervous system. Hence, to determine whether low-dose DPP-4 inhibition could also activate the gut-brain neural axis, we recorded vagus nerve activity before and after an oral glucose load with or without sitagliptin (40 μg/mouse), a dose sufficient to inhibit intestinal but not plasma DPP-4 activity (Fig. 4A). Preliminary experiments using the 4-μg dose did not show any effect that was most likely due to the anesthesia that lowers the sensitivity to the inhibitor. Glucose alone increased the basal firing rate by 2-fold (Fig. 4, B and C). After treatment with sitagliptin, neural activity was further enhanced but could be totally blunted by coadministration of the GLP-1R antagonist Ex9 (Fig. 4, B and C).

The dipeptides His-Ala and Tyr-Ala, generated by degradation of GLP-1 and GIP, modulate glucose tolerance

Inhibition of DPP-4 activity increases the concentration of active incretins in the mesenteric and portal blood but
also reduces the concomitant production of the corresponding degradation products i.e. GLP-19–36 and the dipeptide His-Ala and GIP3–43 and the dipetide Tyr-Ala. Although GLP-19–36 and GIP3–43 have no consistent effect on oral glucose tolerance and insulin secretion, the putative importance of the corresponding dipeptides for the control of glucose tolerance has not been examined. Accordingly, we performed iv (1, 10, and 100 nmol/mouse) injections of the peptides in the presence or absence of a low dose of sitagliptin (40 μg/mouse). Administration of His-Ala alone impaired glucose tolerance (Fig. 5A). Similarly, His-Ala also reduced the glucose regulatory effect of sitagliptin (Fig. 5B). The dipetide reduced plasma insulin levels in the absence of sitagliptin (Fig. 5A, inset) in a dose-dependent manner, whereas a more robust diminution of plasma insulin levels was observed in the presence of sitagliptin (Fig. 5B, inset). His-Ala also impaired glucose tolerance in the absence (Fig. 5C) or presence of sitagliptin (Fig. 5D), together with reduced portal insulin levels (Fig. 5, C and D, insets) and increased levels of portal glucagon (Fig. 5, C and D).
In vitro effect of His-Ala on glucagon and insulin secretion from isolated mouse and human islets

His-Ala was incubated with islets at concentrations ranging from 1 pm to 100 nm, and 100 pm His-Ala reduced glucose-induced insulin secretion from isolated mouse islets (Fig. 6A) and both 1 and 10 nm His-Ala similarly reduced insulin secretion in human islets (Fig. 6C). Conversely, His-Ala dose-dependently increased glucagon secretion in mouse (Fig. 6B) and human (Fig. 6D) islets. In all instances, the basal insulin and glucagon secretion rates remained unchanged by His-Ala.

Discussion

We have now refined our understanding of mechanisms through which DPP-4 inhibitors improve glycemic control. Specifically, we demonstrate the importance of selectively reducing DPP-4 activity in the intestine vs. the systemic circulation, through mechanisms requiring incretin receptors and associated with the triggering of a gut-to-pancreas neural signal. Furthermore, we also identify a putative role for the dipeptides liberated from GLP-1 and GIP by DPP-4 activity.

The importance of the GIP and GLP-1R for transduction of the glucoregulatory actions of DPP-4 inhibitors was revealed through analysis of DPP-4 action in mice with genetic deletion of one or both incretin receptors (33, 34). Similar findings were demonstrated in obese-diabetic ob/ob mice where the glucose-lowering activity of a DPP-4 inhibitor was abolished by coadministration of Ex9 (35). Nevertheless, the relative importance of the intestinal compartment for transduction of a glucoregulatory signal pursuant to local DPP-4 inhibition has not been extensively studied. Our studies were motivated in part by find-
DPP-4 inhibition is likely not the only mechanism responsible for improved glycemic control.

Our data demonstrate efficacy of a very low dose of sitagliptin sufficient to achieve selective intestinal DPP-4 inhibition and glucoregulation without a significant increase in systemic levels of GIP and GLP-1. Hence, activation of glucose-induced insulin secretion by circulating incretins acting directly at the level of the pancreatic β-cell is unlikely to represent a dominant mechanism of action for DPP-4 inhibitor-mediated glucose control. In contrast, much higher doses of the DPP-4 inhibitor were associated with significant increases in circulating active GLP-1, without increased insulin levels likely due to the lower
glycemic excursions and gastric retention of glucose observed in these experiments.

Our findings provide functional support for the importance of DPP-4 localization in capillaries surrounding the enteroendocrine cells within the intestinal mucosa (6). Indeed, lower enterally active doses of sitagliptin did not improve glucose tolerance when administered iv. The local intestinal action of sitagliptin was further supported by our data that show that bioactivity of the inhibitor when administered directly into the portal vein was lower than when administered orally. We cannot rule out that the membrane form of intestinal DPP-4 could have a higher affinity for sitagliptin than the soluble circulating systemic form. Therefore, the efficacy of the inhibitor might be relatively increased in the intestine. This interpretation, however, requires further examination before clear conclusions can be drawn as to how selective inhibition of intestinal DPP-4 preferentially controls glucose metabolism.

The respective importance of GLP-1 and GIP for the actions of DPP-4 inhibitors has previously been delineated in studies using much larger doses of inhibitors producing systemic DPP-4 inhibition (33, 34). Our current data obtained with lower doses of sitagliptin, Ex9, and incretin receptor knockout mice clearly demonstrate an important role for both incretins as mediators of the glucoregulatory mechanisms activated by intestinal DPP-4 inhibition.

We and others have described an enteric role for incretins in the activation of the gut-to-brain-to-periphery axis including the hepatoporal glucose sensor, an important regulator of glucose homeostasis (27, 28, 38). Upon oral absorption of glucose, a positive glucose gradient is established between the mesenteric/hepatoporal vein and the arterial blood. This activates glucose-sensitive structures such as intraganglionic laminar endings in connection with vagus nerve afferents (39–41). These neural structures probably surround the numerous capillaries of the mesenteric veins emptying into the portal vein and are capable of regulating the gut-to-brain neural axis (42, 43). We and others further showed that the GLP-1R is one molecular component of the glucose sensor that regulates many physiological functions involved in the control of glycemia such as muscle glucose utilization, hepatic glucose production, or counterregulatory hormone production (16–21, 44). We first demonstrated that this mechanism increased muscle glucose utilization through an insulin-independent mechanism (28); our current data reveal an imperfect correlation between the effect of sitagliptin to control glucose and changes in portal and plasma insulin levels. Hence, glycemic control achieved by selective intestinal DPP-4 inhibition likely arises in part via an insulin-independent mechanism.

GLP-1R mRNA has been localized to the nodose ganglia, and nerve terminals innervating the portal vein contained immunoreactive GLP-1R (45). The selective blockade of the GLP-1R via hepatoporal infusion of Ex9 partially prevented glucose-induced insulin secretion (46) and the peripheral control of glucose utilization (17). This model, invoking a role for the autonomic nervous system in the gut-to-pancreas axis was supported by experiments using a ganglionic blocker to prevent the insulinotropic action of GLP-1 (47). Our model is consistent with the possibility that the local inhibition of enteric DPP-4 by sitagliptin may be linked to an incretin-dependent signal triggering the enteric gut-to-brain-to-periphery axis proximal to incretin secretion from gut endocrine cells. This hypothesis is supported by our data that show for the first time that sitagliptin increased vagus nerve activity in the basal state and in response to glucose. This mechanism requires an activated GLP-1R because vagus nerve activity was blunted by the coadministration of Ex9.

We previously reported that activation of c-Fos expression in the brain in response to low-dose gastric glucose infusion required the GLP-1R because activation of the
gut-to-brain axis was 1) attenuated by Ex9 infusion and 2) abrogated in Glp1r−/− mice (18). The vagus nerve activity was sensitive to GLP-1 and not to GIP (16, 48), and our current data demonstrate that inhibition of GLP-1 signaling by the coadministration of Ex9 directly into the systemic circulation prevented both the activation of the vagus nerve and the improvement in glycemic control induced by sitagliptin. Taken together, our findings suggest that both incretins trigger glucoregulatory actions locally in the intestine. Although this has been inferred previously for GLP-1, our data suggest for the first time a similar local role of GIP. The mechanism through which GIP acts locally in the gut on glucose homeostasis is unknown and most likely not linked to the regulation of the vagus nerve activity (16, 48). However, it might be related to its role on intestinal glucose absorption by reducing intestinal motility through a somatostatin-mediated pathway as recently described (49).

We have also identified a potential new role for the dipeptides generated from GLP-1 and GIP after cleavage by DPP-4. Inhibition of DPP-4 reduces the production of the GIP- and GLP-1-derived dipeptides Tyr-Ala and His-Ala, respectively. Pharmacological administration of these dipeptides induced glucose intolerance, blunted glucose-induced insulin secretion, and increased glucagon secretion. Moreover, His-Ala exerted direct actions on insulin and glucagon secretion when incubated with isolated murine or human islets. Interestingly, His-Ala alone did not affect basal insulin and glucagon secretion rates, suggesting that the effect of the dipeptide requires concomitant activation of a glucose-sensitive signal in β-cells.

The mechanisms through which dipeptides could regulate insulin and glucagon secretion are not known but could be related to two di- and tripeptide transporters, peptide transporter 1 (PepT1) and PepT2, which have been identified and functionally characterized (50). PepT1 is the low-affinity, high-capacity transporter, whereas PepT2 is the high-affinity, low-capacity transporter and has a broader tissue distribution (51). PepT1 is exclusively expressed in the intestinal epithelial cells, whereas both PepT1 and PepT2 are expressed in the proximal tubule of the kidney. Previous data suggested these dipeptide transporters are present in islets. The oral hypoglycemic agent nateglinide stimulates insulin secretion by inhibiting peptide transport through PepT1 and PepT2 (52). This mechanism would most likely attenuate the incretin effect, which would persist in part through the vagus nerve-dependent gut-brain-pancreatic axis (18, 22). A putative role for dipeptide transporters in the regulation of metabolism has also been suggested because insulin increases PepT1-mediated transport in intestinal cells by recruitment of PepT1 protein from intracellular compart-
Acknowledgments

We thank Gaetan Jougl for excellent technical expertise.

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This work was supported in part by grants from the Merck Sharp and Dohme laboratories to R.B. and D.J.D. and Canadian Institutes for Health Research Grant MOP 82700 to D.J.D. C.M. was recipient of funding from the “gence Nationale de la Recherche ANR-07-PHYSIO, and D.J.D. was supported in part by a Canada Research Chair in Regulatory Peptides and the Novo Nordisk Chair in Incretin Biology.


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