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

Aurélie Waget,* Cendrine Cabou,* Myriam Masseboeuf,* Pierre Cattan, Mattieu Armanet, Mélis Karaca, Julien Castel, Celine Garret, Gaëlle Payros, Adriano Maida, Thierry Sulpice, Jens J. Holst, Daniel J. Drucker, Christophe Magnan, and Rémy Burcelin

Institut de Recherche sur les Maladies Métaboliques et Cardiovasculaires (I2MC) de l'Hôpital Rangueil, (A.W., C.C., M.M., C.G., G.P., R.B.), Unité 1048 Institut National de la Santé et de la Recherche Médicale (Inserm), 31400 Toulouse, France; Department of Medicine (A.M., D.J.D.), Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, M5G2C4 Toronto, Ontario, Canada; Unit of Functional and Adaptative Biology (M.K., J.C., C.M.), Centre National de la Recherche Scientifique (CNRS) 4413, 75013 Paris, France; Cell Therapy Unit (P.C., M.A.), Hôpital Saint Louis, Assistance Publique-Hôpitaux de Paris, and University Paris 7, 75010 Paris, France; Inserm U872 (P.C., M.A.). Centre de recherches des Cordeliers, 75006 Paris, France; Department of Biomedical Sciences (J.J.H.), The Panum Institute, University of Copenhagen, DK2200 Copenhagen, Denmark; and Physiogenex SAS (T.S.), Prologue Biotech, 31682 Labège Innopole, France

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: 3018–3029, 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-

ISSN Print 0013-7227 ISSN Online 1945-7170
Printed in U.S.A.

doi: 10.1210/en.2011-0286 Received March 15, 2011. Accepted May 24, 2011.
First Published Online June 14, 2011

For editorial see page 2925

* A.W., C.C., and M.M. contributed equally to this work.

Abbreviations: DPP-4, Dipeptidyl peptidase-4; Ex9, exendin (9–39); GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide 1; GLP-1R, GLP-1 receptor; PepT1, peptide transporter 1.

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 glycemic 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 hepatoportal 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 enteroportal 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 C57BL6/J (Charles River, L'Arbresle, France), *Glp1r*$^{-/-}$ and *Gipr*$^{-/-}$ 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-1Rantagonist 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 koctet/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 handpicking 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 25 mM HEPES at 37 C in 95% O_2/5% CO_2 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 nM, 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/m^2 < body mass index < 25 kg/m^2) with brain death as previously described (31). The islets were handpicked 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 Na_2HPO_4,1.5 mM KH_2PO_4) 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 Bonferoni *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 glucoregulation (Fig. 1, B and C). Interestingly, a pharmacological dose of 40 mg almost totally blunted glycemic excursion (not shown)

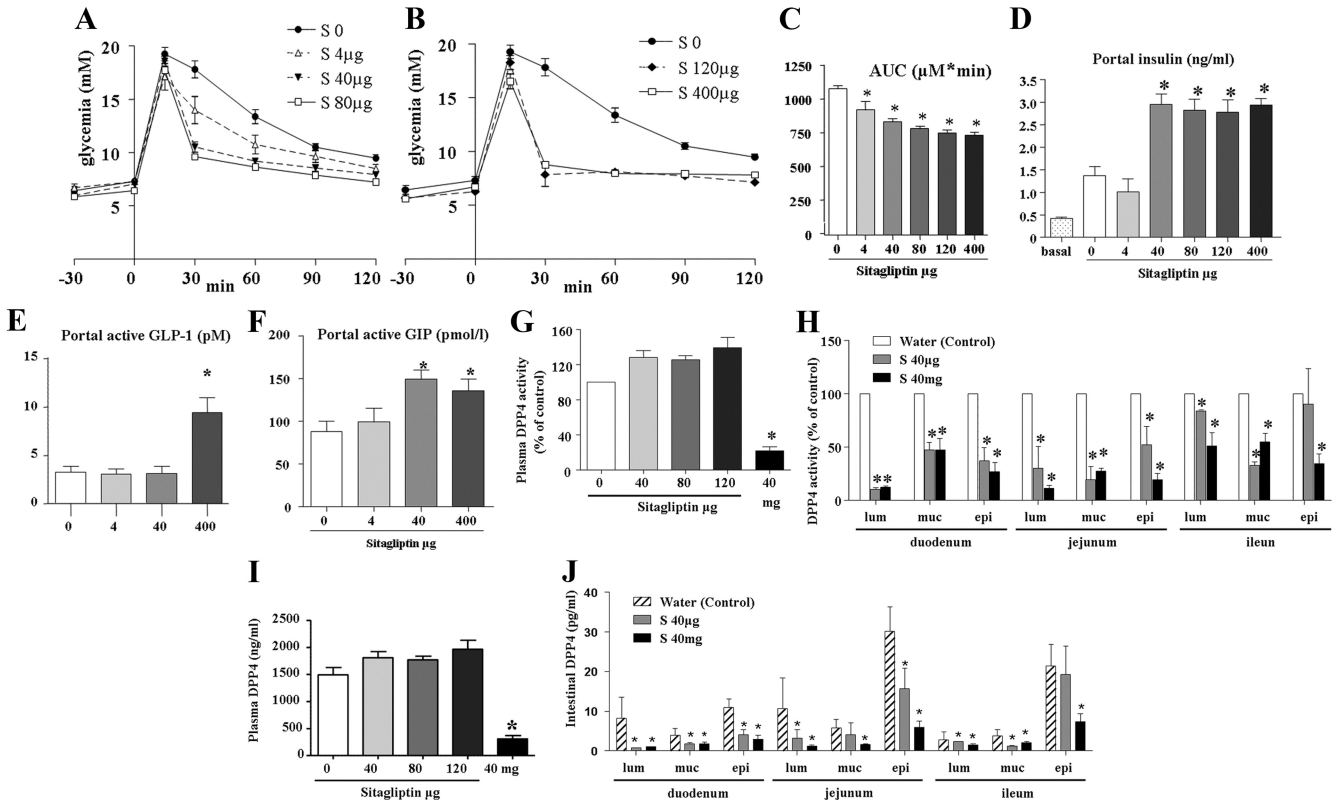

FIG. 1. Oral administration of sitagliptin. In all instances, sitagliptin was administered orally 30 min before the oral glucose challenge. A and B, Glycemic profiles 30 min before and during an oral glucose tolerance test (n = 12 for 0, 4, 40, and 80 μg/mouse, and n = 8 for 120 and 400 μg/mouse); C, corresponding area under curve (AUC) (micromolar × minute) for data in A and B; D, portal concentrations of plasma insulin (nanograms per milliliter) measured in different sets of mice: E, portal active GLP-1 (picomolar); F, portal active GIP (picomoles per liter); G–J, DPP-4 activity (percentage of control in the absence of sitagliptin) (G and H) and concentration (picograms per milliliter) (I and J) in the plasma (G and I), and in the different segments of the intestinal lumen (lum), mucosa (muc), or in the intestinal submucosal epithelium (epi) (H and J) determined 15 min after the glucose challenge in mice where sitagliptin at the dose of 40 μg/mouse or 40 mg/mouse has been administered orally. *, Statistically significant from the non-sitagliptin-treated group, $P < 0.05$.

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). In contrast, the control dose of 40 mg sitagliptin reduced DPP-4 activity in all compartments (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 sitapliptin 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–

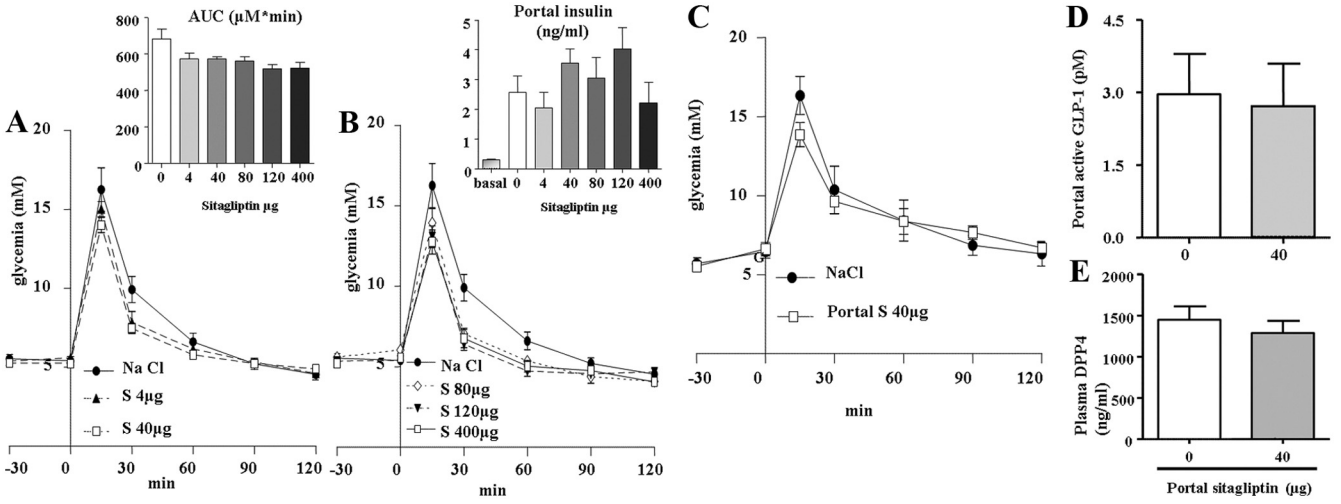

FIG. 2. Intravenous sitagliptin administration. In all instances, sitagliptin was administered iv 30 min before the oral glucose challenge. A and B, Glycemic profiles 30 min before and during oral glucose tolerance test after iv administration of sitagliptin (n = 12 for 0, 4, 40, and 80 µg/mouse, and n = 6 for 120 and 400 µg/mouse); *inset* in A, corresponding area under curve (AUC) are represented in micromolar per minute for data shown in A and B. Portal hormone levels were measured indifferent sets of mice. *Inset* in B, Portal concentrations of plasma insulin (nanograms per milliliter) 15 min after glucose administration (six mice per group) (see Supplemental Fig. 1 for the corresponding DPP-4 activities). C, Glycemic profiles during oral glucose tolerance test after portal vein administration of sitagliptin (40 µg/mouse, six mice per group). D, Portal active GLP-1 concentrations (picomolar) and E, circulating DPP-4 activity (nanograms per milliliter) in mice where 40 µg/mouse of sitagliptin or NaCl (0.9%) has been injected into the portal vein (five mice per group).

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 at the lowest maximally efficient intestinal glucoregulatory dose of 40 µg/mouse. Ex9 impaired the antihyperglycemic action of low-dose sitagliptin (Fig. 3A). A similar effect was observed with sitagliptin administered at the dose of 80 µg/mouse (not shown). Surprisingly, Ex9 did not block the increase in plasma insulin levels previously observed (Fig. 1D) with 40 µg/mouse sitagliptin (Fig. 3B). To further study the role of incretin receptors in the control of glycemia by low-dose sitagliptin, we analyzed $Glp1r^{-/-}$ and $Gipr^{-/-}$ mice. Sitagliptin at doses of 4, 40, or 120 µg/mouse had no effect on oral glucose tolerance in $Glp1r^{-/-}$ mice, whereas the 120-µg dose did improve glycemic profiles (Fig. 3C). Plasma insulin levels increased modestly after sitagliptin treatment (Fig. 3, C and D). Similarly, whereas the two lower doses, 4 and 40 µg/mouse sitagliptin, had no effect on glucose excursion or insulin levels, 120 µg sitagliptin did reduce glucose excursions in $Gipr^{-/-}$ mice (Fig. 3D).

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

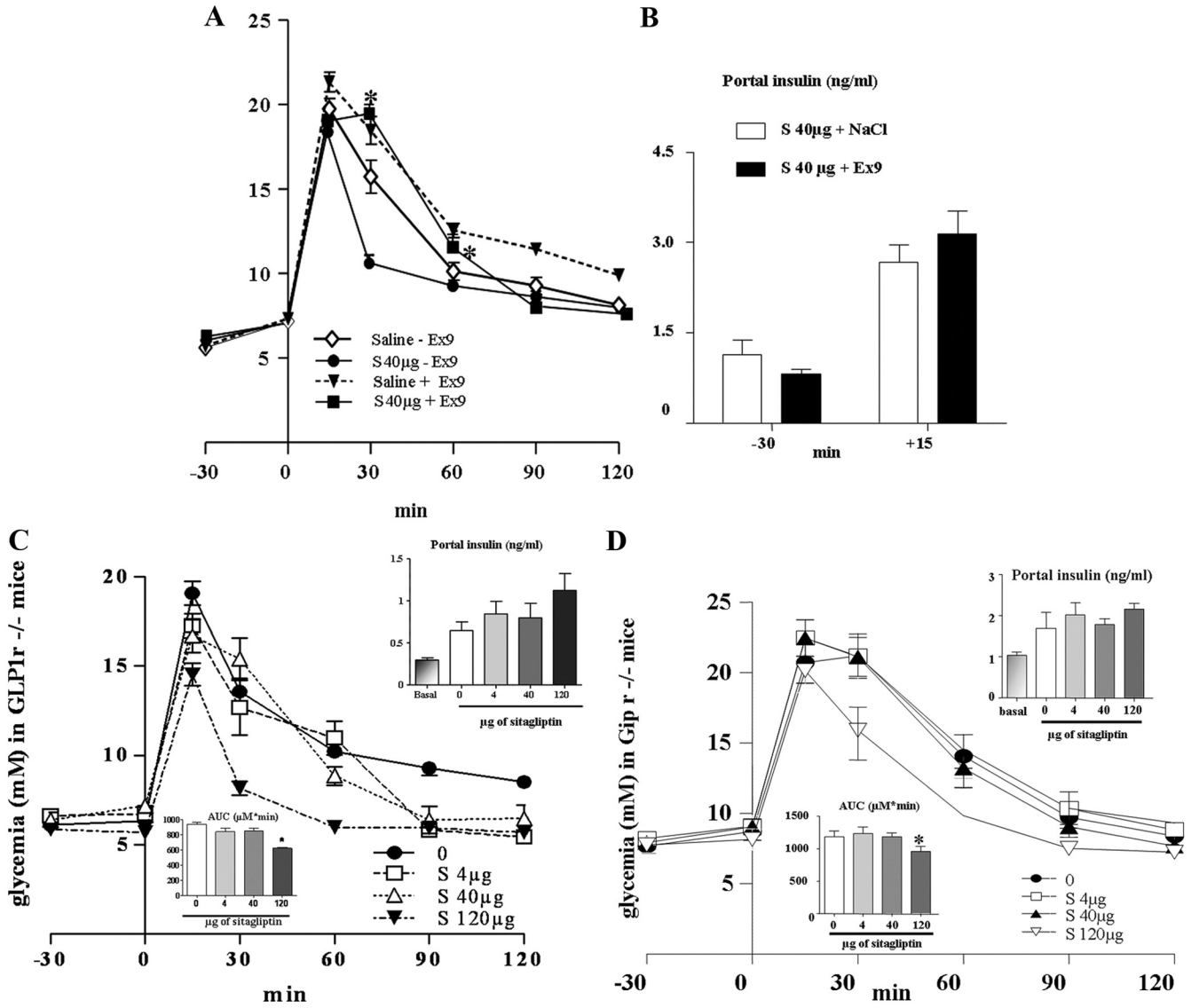

FIG. 3. Sitagliptin action after transient or genetic disruption of incretin receptor signaling. A, Oral glucose tolerance assessed in wild-type mice in the presence or absence of Ex9 after low-dose sitagliptin (S), 40 μg/mouse, six mice per group or saline administered orally 30 min before the oral glucose challenge; B, portal concentrations of plasma insulin after 40 μg/mouse sitagliptin in the presence or absence of Ex9 (six mice per group); C, effects of sitagliptin, 4, 40, and 120 μg/mouse (six mice per group) on oral glucose tolerance in *Glp1r*$^{-/-}$ mice; *insets* in C, corresponding AUC (micromolar per minute; *lower inset*) for glycemia and for portal plasma insulin values (nanograms per milliliter; *higher inset*) 15 min after the glucose challenge in *Glp1r*$^{-/-}$ mice; D, effects of sitagliptin, 4, 40, or 120 μg/mouse (ten, nine, and eight mice per group, respectively) on glucose tolerance in *Gipr*$^{-/-}$ mice; *insets* in D, corresponding area under the curve (AUC) (micromolar per minute; *lower inset*) for glycemia and for portal insulin values (nanograms per milliliter; *higher inset*) 15 min after the glucose challenge in *Gipr*$^{-/-}$ mice. *, Statistically significant from control, $P < 0.05$.

also reduces the concomitant production of the corresponding degradation products *i.e.* GLP-1_{9-36} and the dipeptide His-Ala and GIP_{3-43} and the dipetide Tyr-Ala. Although GLP-1_{9-36} and GIP_{3-43} 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 glucoregulatory effect of sitagliptin (Fig. 5B). The dipeptide 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 increased portal glucagon levels after oral glucose challenge (Fig. 5A, *inset*). Importantly, Tyr-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).

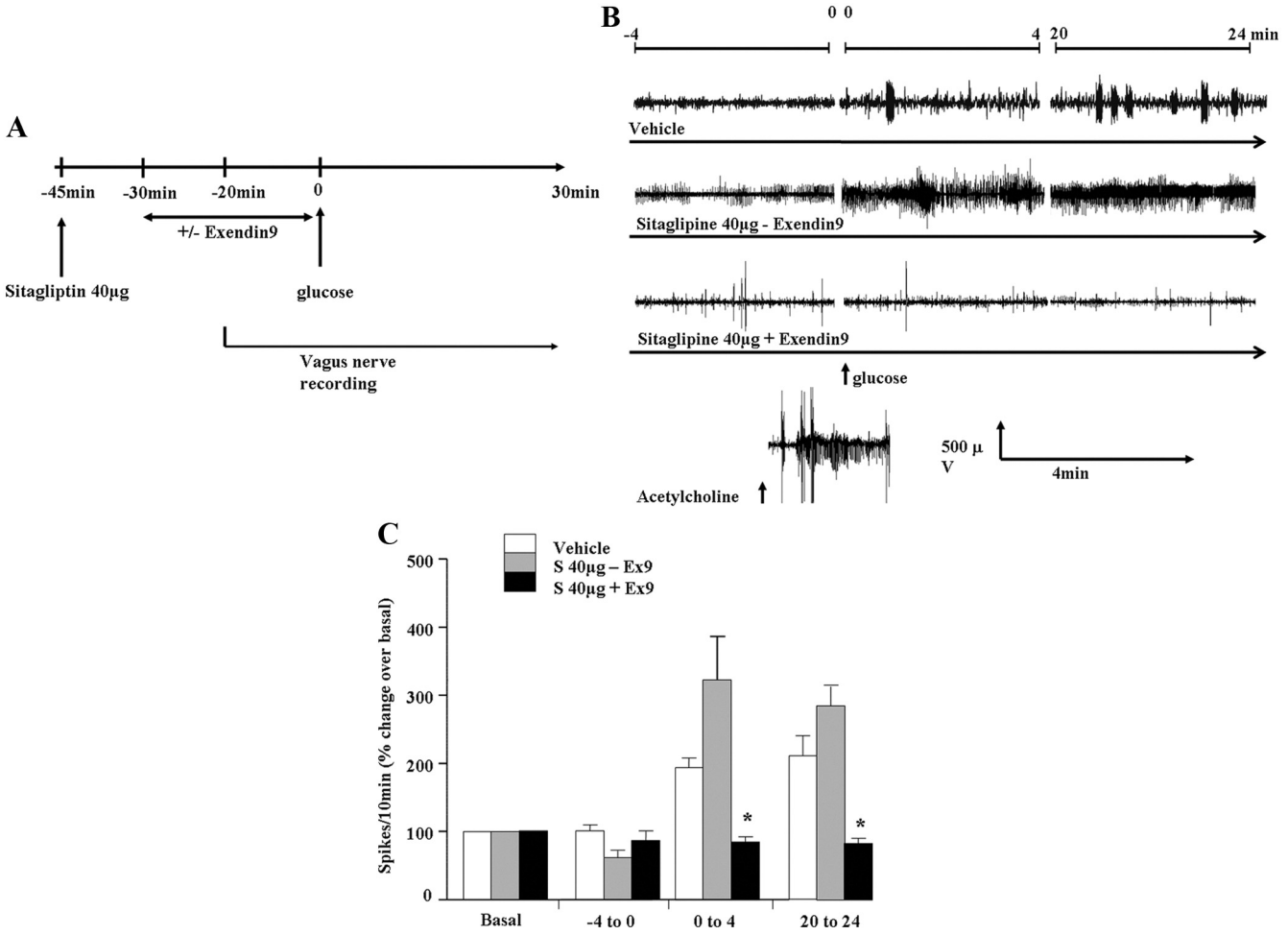

FIG. 4. Effect of sitagliptin and Ex9 on glucose-induced vagus nerve activation. A, Experimental design: 45 min before the glucose challenge, 40 μg sitagliptin or water was administered orally to the mice, and then Ex9 was infused in the femoral vein at the rate of 0.1 nmol/kg·min 30 min before the glucose challenge, after which glucose was directly administered into the stomach. B, The vagus nerve activity (millivolts) has been recorded before and after the glucose challenge (*upper panel*) for up to 24 min or in mice where sitagliptin has been administered in the absence of Ex9 (*middle panel*) or the presence (*lower panel*) of Ex9. After completion of the recording, 600 μl acetylcholine was administered ip to validate the specific recording. C, Quantification of the number of spikes per 10 min in percentage of change over basal (before glucose challenge). *, Statistically significant from the group of mice not treated with sitagliptin, $P < 0.05$ with four mice per group studied.

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-

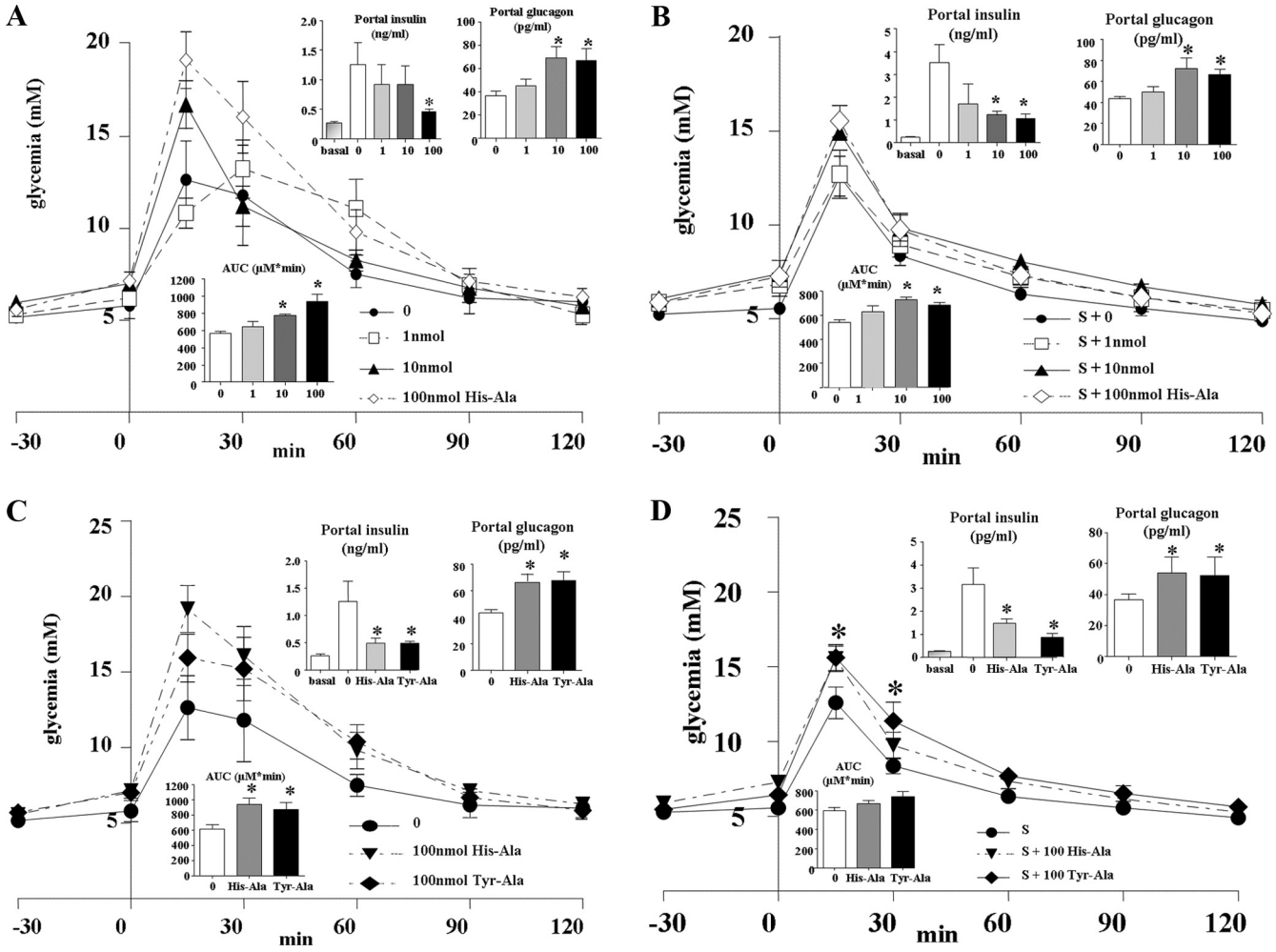

FIG. 5. The incretin dipeptide degradation products His-Ala and Tyr-Ala blunt the glucoregulatory action of sitagliptin. A and B, Glycemic profiles before (basal) and during oral glucose tolerance tests in mice treated with 1, 10, 100 nmol of His-Ala (iv). Water (A) (eight mice per group) or sitagliptin (B) (eight mice per group) at the dose of 40 μg/mouse was administered orally 30 min before the glucose challenge. *Insets* in A and B, Corresponding area under the curve (AUC) (micromolar per minute; *lower inset*) for glycemia. In different sets of mice, portal plasma insulin (nanograms per milliliter; *higher inset left*) and portal glucagon (picograms per milliliter, *higher inset right*) values corresponding to oral glucose tolerance test analyses are reported 15 min after the glucose challenge. C and D, glycemic profiles before (basal) and during oral glucose tolerance tests in mice treated with 100 nmol His-Ala (eight mice per group) or Tyr-Ala (eight mice per group) in the absence (C) or presence (D) of 40 μg/mouse oral sitagliptin; *insets* in C and D, corresponding AUC (micromolar per minute; *lower inset*) for glycemia, for portal plasma insulin (nanograms per milliliter; *higher inset left*), and for portal glucagon (picograms per milliliter, *higher inset right*) values corresponding to oral glucose tolerance test analyses 15 min after the glucose challenge. *, Statistically significant from the group of mice not treated with dipeptides, $P < 0.05$.

ings that the acute oral or systemic administration of DPP-4 inhibitors produces only modest increases in circulating active incretin and insulin levels in animal models of diabetes (36). Similarly, in humans, augmentation of plasma levels of active GLP-1 with DPP-4 inhibition is nonlinear and maximized at 10 mg of PF-00734200, a dose that resulted in about 75% weighted average DPP-4 inhibition over 24 h and only a 2.3-fold increase in active GLP-1 levels over placebo (37). Moreover, whereas substantial inhibition of plasma DPP-4 activity was observed for over 24 h, plasma GLP-1 concentrations declined over the same time period (37). Hence, a transient increase in circulating plasma GLP-1 concentrations associated with 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

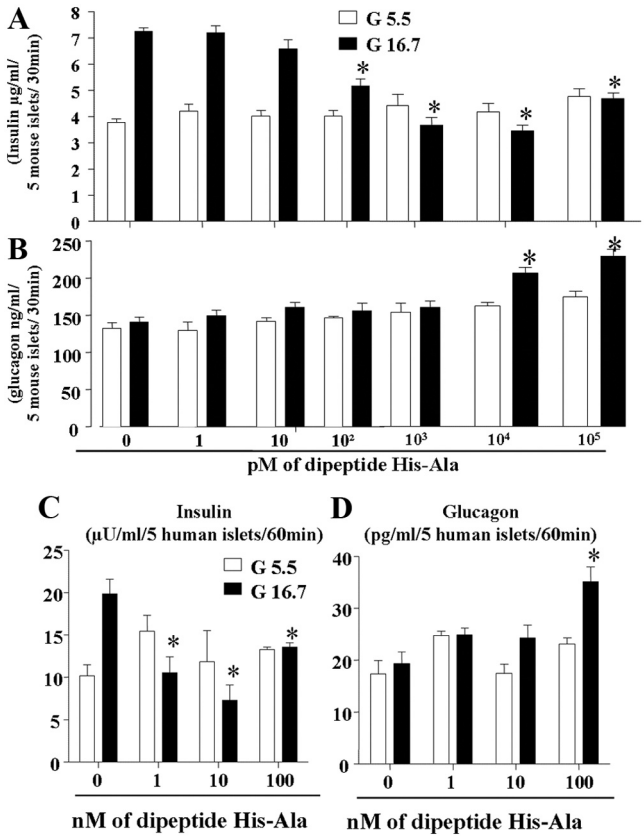

FIG. 6. His-Ala regulates insulin and glucagon secretion in human and mouse islets. Measurement of insulin and glucagon secretion from isolated C57BL6 mouse (A and B) and human (C and D) islets in the absence (−) or presence of His-Ala at the indicated concentrations and with basal (G5.5 mM) or high (G16.7 mM) glucose concentration. *, $P < 0.05$ when compared without His-Ala; n = 6 replicates per well.

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 hepatoportal 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/hepatoportal 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 hepatoportal 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 compartments into the membrane (53). Rats made diabetic also demonstrated increased PepT1 activity and protein (54). Interestingly, these dipeptide transporters have a strong proinflammatory effect because they are able to transport formyl-Met-Leu-Phe, a known neutrophil attractant peptide derived from certain bacterial species linked with colonic inflammation (55).

PepT1 is expressed at higher levels in inflamed colonic tissues, whereas in healthy adult colon, it is not expressed (56). By preventing the release of dipeptides and PepT1 proinflammatory actions, sitagliptin might lower low-grade inflammation as recently suggested (57), which could contribute to improvement of the gut-brain axis. Therefore, reduction of dipeptide release from the degradation of the incretins, or other peptides from alimentary origin, could contribute to the regulation of insulin and glucagon secretion under appropriate concentrations and conditions. The findings that the dipeptide products generated by DPP-4 have biological actions further support the growing understanding that these endopeptidases are not simply involved in the degradation of the hormones into inactive metabolites, but rather may also generate peptide metabolites with new biological activities.

Our hypothesis is that DPP-4-mediated incretin degradation along with the production of other dipeptides liberated during a meal by the endopeptidase activity of the luminal and enteric DPP-4 would produce a sufficient concentration of dipeptides to regulate glucagon secretion. Our observation is also consistent with previous findings that liberation of dipeptides after ingestion of a protein-enriched diet may also inhibit local DPP-4 activity and increase bioactive GLP-1 in the intestine (58), thereby regulating food intake and glycemia (59).

Taken together, our data strongly suggest that DPP-4 inhibitors control postprandial glycemia in part via inhibition of intestinal DPP-4 proximal to the site of GLP-1 secretion. This would lead to activation of the neural gut-to-brain-to-β-cell axis through a mechanism that does not require direct actions of circulating GLP-1 on islet cells. Rather, the dominant mechanism through which low-dose DPP-4 inhibition controls glycemia may involve enteric GLP-1 signaling as a component of the gut-to-β-cell axis. Furthermore, we provide new evidence suggesting a potential biological role for bioactive dipeptides whose concentrations are regulated by DPP-4 activity. Hence, our findings highlight emerging differences in the mechanism of action of DPP-4 inhibitors distinct from those observed with GLP-1R agonists and emphasize the increasing importance of gut-derived signals in the control of glucose homeostasis.

Acknowledgments

We thank Gaetan Jougla for excellent technical expertise.

Address all correspondence and requests for reprints to: Rémy Burcelin, I2MC, Institut de Recherche sur les Maladies Métaboliques et Cardiovasculaires de l'Hôpital Rangueil, Inserm U1048, BP 84225, 31432 Toulouse Cedex 4, France. E-mail: remy.burcelin@inserm.fr.

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.

A.W., C.C., M.M., P.C., M.A., M.K., J.C., C.G., G.P., A.M., and J.J. carried out the experiments; D.J.D., T.S., J.J., C.M., and R.B. analyzed the data; and C.M., D.J.D., and R.B. wrote the paper.

Disclosure Summary: A.W., C.C., M.M., P.C., M.A., M.K., J.C., C.G., G.P., A.M., C.M., and R.B. declare that they have no conflict of interest. D.J.D. has served as a consultant for Merck Inc. T.S. is an employee of Physiogenex SAS.

References

1. **Holst JJ** 2007 The physiology of glucagon-like peptide 1. Physiol Rev 87:1409–1439
2. **Burcelin R** 2005 The incretins: a link between nutrients and well-being. Br J Nutr 93(Suppl 1):S147–S156
3. **Weir GC, Mojsov S, Hendrick GK, Habener JF** 1989 Glucagonlike peptide I (7–37) actions on endocrine pancreas. Diabetes 38:338–342
4. **Thorens B, Waeber G** 1993 Glucagon-like peptide-I and the control of insulin secretion in the normal state and in NIDDM. Diabetes 42:1219–1225
5. **Creutzfeldt W** 1979 The incretin concept today. Diabetologia 16: 75–85
6. **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
7. **Deacon CF** 2004 Circulation and degradation of GIP and GLP-1. Horm Metab Res 36:761–765
8. **Marguet D, Baggio L, Kobayashi T, Bernard AM, Pierres M, Nielsen PF, Ribel U, Watanabe T, Drucker DJ, Wagtmann N** 2000 Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. Proc Natl Acad Sci USA 97:6874–6879
9. **Elahi D, Andersen DK, Brown JC, Debas HT, Hershcopf RJ, Raizes GS, Tobin JD, Andres R** 1979 Pancreatic α- and β-cell responses to GIP infusion in normal man. Am J Physiol 237:E185–E191
10. **O'Harte FP, Mooney MH, Flatt PR** 1999 NH_2-terminally modified gastric inhibitory polypeptide exhibits amino-peptidase resistance and enhanced antihyperglycemic activity. Diabetes 48:758–765
11. **Widenmaier SB, Kim SJ, Yang GK, De Los Reyes T, Nian C, Asadi A, Seino Y, Kieffer TJ, Kwok YN, McIntosh CH** 2010 A GIP receptor agonist exhibits β-cell anti-apoptotic actions in rat models of diabetes resulting in improved β-cell function and glycemic control. PLoS One 5:e9590
12. **Degn KB, Juhl CB, Sturis J, Jakobsen G, Brock B, Chandramouli V, Rungby J, Landau BR, Schmitz O** 2004 One week's treatment with the long-acting glucagon-like peptide 1 derivative liraglutide (NN2211) markedly improves 24-h glycemia and α- and β-cell function and reduces endogenous glucose release in patients with type 2 diabetes. Diabetes 53:1187–1194
13. **Gedulin BR, Nikoulina SE, Smith PA, Gedulin G, Nielsen LL, Baron AD, Parkes DG, Young AA** 2005 Exenatide (exendin-4) improves insulin sensitivity and β-cell mass in insulin-resistant obese fa/fa Zucker rats independent of glycemia and body weight. Endocrinology 146:2069–2076
14. **Silvestre RA, Rodríguez-Gallardo J, Egido EM, Marco J** 2003 Interrelationship among insulin, glucagon and somatostatin secretory responses to exendin-4 in the perfused rat pancreas. Eur J Pharmacol 469:195–200
15. **EuCSGLP1, Burcelin R** 2008 What is known, new and controversial about GLP-1? Diabetes Metab 34:627–630
16. **Nakabayashi H, Nishizawa M, Nakagawa A, Takeda R, Niijima A** 1996 Vagal hepatopancreatic reflex effect evoked by intraportal appearance of tGLP-1. Am J Physiol 271:E808–E813
17. **Burcelin R, Da Costa A, Drucker D, Thorens B** 2001 Glucose competence of the hepatoportal vein sensor requires the presence of an activated glucagon-like peptide-1 receptor. Diabetes 50:1720–1728
18. **Knauf C, Cani PD, Kim DH, Iglesias MA, Chabo C, Waget A, Colom A, Rastrelli S, Delzenne NM, Drucker DJ, Seeley RJ, Burcelin R** 2008 Role of central nervous system glucagon-like peptide-1 receptors in enteric glucose sensing. Diabetes 57:2603–2612
19. **Dardevet D, Moore MC, Neal D, DiCostanzo CA, Snead W, Cherrington AD** 2004 Insulin-independent effects of GLP-1 on canine liver glucose metabolism: duration of infusion and involvement of hepatoportal region. Am J Physiol Endocrinol Metab 287:E75–E81
20. **Johnson KM, Edgerton DS, Rodewald T, Scott M, Farmer B, Neal D, Cherrington AD** 2007 Intraportal GLP-1 infusion increases nonhepatic glucose utilization without changing pancreatic hormone levels. Am J Physiol Endocrinol Metab 293:E1085–E1091
21. **Johnson KM, Edgerton DS, Rodewald T, Scott M, Farmer B, Neal D, Cherrington AD** 2008 Intraportally delivered GLP-1, in the presence of hyperglycemia induced via peripheral glucose infusion, does not change whole body glucose utilization. Am J Physiol Endocrinol Metab 294:E380–E384
22. **Knauf C, Cani PD, Perrin C, Iglesias MA, Maury JF, Bernard E, Benhamed F, Grémeaux T, Drucker DJ, Kahn CR, Girard J, Tanti JF, Delzenne NM, Postic C, Burcelin R** 2005 Brain glucagon-like peptide-1 increases insulin secretion and muscle insulin resistance to favor hepatic glycogen storage. J Clin Invest 115:3554–3563
23. **Cabou C, Campistron G, Marsollier N, Leloup C, Cruciani-Guglielmacci C, Pénicaud L, Drucker DJ, Magnan C, Burcelin R** 2008 Brain GLP-1 regulates arterial blood flow, heart rate and insulin sensitivity. Diabetes 57:2577–2587
24. **Sandoval DA, Bagnol D, Woods SC, D'Alessio DA, Seeley RJ** 2008 Arcuate GLP-1 receptors regulate glucose homeostasis but not food intake. Diabetes 57:2046–2054
25. **Duez H, Smith AC, Xiao C, Giacca A, Szeto L, Drucker DJ, Lewis GF** 2009 Acute dipeptidyl peptidase-4 inhibition rapidly enhances insulin-mediated suppression of endogenous glucose production in mice. Endocrinology 150:56–62
26. **Burcelin R, Cani PD, Knauf C** 2007 Glucagon-like peptide-1 and energy homeostasis. J Nutr 137:2534S–2538S
27. **Burcelin R, Dolci W, Thorens B** 2000 Glucose sensing by the hepatoportal sensor is GLUT-2 dependent: in vivo analysis in GLUT-2null mice. Diabetes 49:1643–1648
28. **Burcelin R, Dolci W, Thorens B** 2000 Portal glucose infusion in the mouse induces hypoglycemia. Evidence that the hepatoportal glucose sensor stimulates glucose utilization. Diabetes 49:1635–1642
29. **Deacon CF, Nauck MA, Meier J, Hücking K, Holst JJ** 2000 Degradation of endogenous and exogenous gastric inhibitory polypeptide in healthy and in type 2 diabetic subjects as revealed using a new assay for the intact peptide. J Clin Endocrinol Metab 85:3575–3581
30. **Cabou C, Cani PD, Campistron G, Knauf C, Mathieu C, Sartori C, Amar J, Scherrer U, Burcelin R** 2007 Central insulin regulates heart

rate and arterial blood flow: an endothelial nitric oxide synthase-dependent mechanism altered during diabetes. Diabetes 56:2872–2877

31. **Van De Winkel M, Pipeleers D** 1983 Autofluorescence-activated cell sorting of pancreatic islet cells: purification of insulin-containing B-cells according to glucose-induced changes in cellular redox state. Biochem Biophys Res Commun 114:835–842
32. **Knauf C, Cani PD, Ait-Belgnaoui A, Benani A, Dray C, Cabou C, Colom A, Uldry M, Rastrelli S, Sabatier E, Godet N, Waget A, Pénicaud L, Valet P, Burcelin R** 2008 Brain glucagon-like peptide 1 signaling controls the onset of high-fat diet-induced insulin resistance and reduces energy expenditure. Endocrinology 149:4768–4777
33. **Hansotia T, Baggio LL, Delmeire D, Hinke SA, Yamada Y, Tsukiyama K, Seino Y, Holst JJ, Schuit F, Drucker DJ** 2004 Double incretin receptor knockout (DIRKO) mice reveal an essential role for the enteroinsular axis in transducing the glucoregulatory actions of DPP-IV inhibitors. Diabetes 53:1326–1335
34. **Flock G, Baggio LL, Longuet C, Drucker DJ** 2007 Incretin receptors for glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide are essential for the sustained metabolic actions of vildagliptin in mice. Diabetes 56:3006–3013
35. **Duffy NA, Green BD, Irwin N, Gault VA, McKillop AM, O'Harte FP, Flatt PR** 2007 Effects of antidiabetic drugs on dipeptidyl peptidase IV activity: nateglinide is an inhibitor of DPP IV and augments the antidiabetic activity of glucagon-like peptide-1. Eur J Pharmacol 568:278–286
36. **Moritoh Y, Takeuchi K, Asakawa T, Kataoka O, Odaka H** 2008 Chronic administration of alogliptin, a novel, potent, and highly selective dipeptidyl peptidase-4 inhibitor, improves glycemic control and β-cell function in obese diabetic ob/ob mice. Eur J Pharmacol 588:325–332
37. **Dai H, Gustavson SM, Preston GM, Eskra JD, Calle R, Hirshberg B** 2008 Non-linear increase in GLP-1 levels in response to DPP-IV inhibition in healthy adult subjects. Diabetes Obes Metab 10:506–513
38. **Niijima A** 1969 Afferent impulse discharges from glucoreceptors in the liver of the guinea pig. Ann NY Acad Sci 157:690–700
39. **Berthoud HR, Kressel M, Neuhuber WL** 1992 An anterograde tracing study of the vagal innervation of rat liver, portal vein and biliary system. Anat Embryol 186:431–442
40. **Berthoud HR, Niijima A, Sauter JF, Jeanrenaud B** 1983 Evidence for a role of the gastric, coeliac and hepatic branches in vagally stimulated insulin secretion in the rat. Journal of the Autonomic Nervous System 7:97–110
41. **Berthoud HR, Patterson LM, Neumann F, Neuhuber WL** 1997 Distribution and structure of vagal afferent intraganglionic laminar endings (IGLEs) in the rat gastrointestinal tract. Anat Embryol (Berl) 195:183–191
42. **Niijima A** 1981 Neurophysiological evidence for hepatic glucose-sensitive afferents. Commentary on "The current status of hepatic theory of food intake control." Appetite 2:151–152
43. **Niijima A** 1982 Glucose-sensitive afferent nerve fibres in the hepatic branch of the vagus nerve in the guinea-pig. J Physiol 332:315–323
44. **Ionut V, Hucking K, Liberty IF, Bergman RN** 2005 Synergistic effect of portal glucose and glucagon-like peptide-1 to lower systemic glucose and stimulate counter-regulatory hormones. Diabetologia 48:967–975
45. **Vahl TP, Tauchi M, Durler TS, Elfers EE, Fernandes TM, Bitner RD, Ellis KS, Woods SC, Seeley RJ, Herman JP, D'Alessio DA** 2007 Glucagon-like peptide-1 (GLP-1) receptors expressed on nerve terminals in the portal vein mediate the effects of endogenous GLP-1 on glucose tolerance in rats. Endocrinology 148:4965–4973
46. **Preitner F, Ibberson M, Franklin I, Binnert C, Pende M, Gjinovci A, Hansotia T, Drucker DJ, Wollheim C, Burcelin R, Thorens B** 2004 Gluco-incretins control insulin secretion at multiple levels as revealed in mice lacking GLP-1 and GIP receptors. J Clin Invest 113:635–645
47. **Balkan B, Li X** 2000 Portal GLP-1 administration in rats augments the insulin response to glucose via neuronal mechanisms. Am J Physiol Regul Integr Comp Physiol 279:R1449–R1454
48. **Nishizawa M, Nakabayashi H, Uchida K, Nakagawa A, Niijima A** 1996 The hepatic vagal nerve is receptive to incretin hormone glucagon-like peptide-1, but not to glucose-dependent insulinotropic polypeptide, in the portal vein. J Auton Nerv Syst 61:149–154
49. **Ogawa E, Hosokawa M, Harada N, Yamane S, Hamasaki A, Toyoda K, Fujimoto S, Fujita Y, Fukuda K, Tsukiyama K, Yamada Y, Seino Y, Inagaki N** 2011 The effect of gastric inhibitory polypeptide on intestinal glucose absorption and intestinal motility in mice. Biochem Biophys Res Commun 404:115–120
50. **Nielsen CU, Brodin B** 2003 Di/tri-peptide transporters as drug delivery targets: regulation of transport under physiological and patho-physiological conditions. Curr Drug Targets 4:373–388
51. **Rubio-Aliaga I, Daniel H** 2008 Peptide transporters and their roles in physiological processes and drug disposition. Xenobiotica 38:1022–1042
52. **Terada T, Sawada K, Saito H, Hashimoto Y, Inui K** 2000 Inhibitory effect of novel oral hypoglycemic agent nateglinide (AY4166) on peptide transporters PEPT1 and PEPT2. Eur J Pharmacol 392:11–17
53. **Thamotharan M, Bawani SZ, Zhou X, Adibi SA** 1999 Hormonal regulation of oligopeptide transporter pept-1 in a human intestinal cell line. Am J Physiol 276:C821–C826
54. **Gangopadhyay A, Thamotharan M, Adibi SA** 2002 Regulation of oligopeptide transporter (Pept-1) in experimental diabetes. Am J Physiol Gastrointest Liver Physiol 283:G133–G138
55. **Buyse M, Berlioz F, Guilmeau S, Tsocas A, Voisin T, Péranzi G, Merlin D, Laburthe M, Lewin MJ, Rozé C, Bado A** 2001 PepT1-mediated epithelial transport of dipeptides and cephalexin is enhanced by luminal leptin in the small intestine. J Clin Invest 108:1483–1494
56. **Merlin D, Si-Tahar M, Sitaraman SV, Eastburn K, Williams I, Liu X, Hediger MA, Madara JL** 2001 Colonic epithelial hPepT1 expression occurs in inflammatory bowel disease: transport of bacterial peptides influences expression of MHC class 1 molecules. Gastroenterology 120:1666–1679
57. **Takasawa W, Ohnuma K, Hatano R, Endo Y, Dang NH, Morimoto C** 2010 Inhibition of dipeptidyl peptidase 4 regulates microvascular endothelial growth induced by inflammatory cytokines. Biochem Biophys Res Commun 401:7–12
58. **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
59. **Pillot B, Soty M, Gautier-Stein A, Zitoun C, Mithieux G** 2009 Protein feeding promotes redistribution of endogenous glucose production to the kidney and potentiates its suppression by insulin. Endocrinology 150:616–624