Acute Dipeptidyl Peptidase-4 Inhibition Rapidly Enhances Insulin-Mediated Suppression of Endogenous Glucose Production in Mice

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Pharmacological approaches that enhance incretin action for the treatment of type 2 diabetes mellitus have recently been developed, *i.e.* injectable glucagon-like peptide-1 receptor (GLP-1R) agonists with prolonged plasma half-lives and orally available inhibitors of dipeptidyl peptidase (DPP)-4, the main enzyme responsible for the rapid degradation of circulating glucagon-like peptide-1 and glucose-dependent insulinotropic peptide. The mechanism(s) underlying the glucose-lowering effect of these two pharmacotherapies differs and is not yet fully understood. Here we investigated whether acute GLP-1R activation (exendin-4) or DPP-4 inhibition (des-F-sitagliptin) modulates insulin action in mice using a hyperinsulinemic euglycemic clamp. A single iv bolus of des-F-sitagliptin (11 mg/kg) was administered to mice 15 min after the start of the clamp, and its effect was compared with a 50-ng bolus of exendin-4 or the same volume of saline. Despite matched levels of plasma glucose and insulin, within 15 min the glucose infusion rate required to maintain euglycemia was significantly greater after des-F-sitagliptin compared with saline or exendin-4. This difference was entirely due to enhancement of insulin-mediated suppression of endogenous glucose production by des-F-sitagliptin, with no difference in glucose disposal rate. These findings illustrate that DPP-4 inhibition modulates glucose homeostasis through pathways distinct from those used by GLP-1R agonists in mice. **(*Endocrinology* 150: 56–62, 2009)**

Diabetes affects an increasing number of individuals and has now assumed epidemic proportions. Promising pharmacological approaches recently approved for the treatment of type 2 diabetes include two incretin-based therapies, the glucagon-like peptide-1 receptor (GLP-1R) agonists or molecules that inhibit the breakdown of incretin hormones [*i.e.* dipeptidyl peptidase (DPP)-4 inhibitors] (1). Incretin-based therapies exert their insulinotropic effect in a glucose-dependent fashion, thereby limiting the occurrence of hypoglycemia. These agents may also either induce weight loss (GLP-1R agonists) or are not associated with weight gain (DPP-4 inhibitors), in contrast to findings with other presently available treatments for type 2 diabetes (1).

Glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic peptide (GIP) are the two principal hormones acting as incretins, which are released by the intestine and act on pancreatic islets to potentiate glucose-stimulated insulin secretion (GSIS) (2, 3). Exogenous infusion of GLP-1 or a potent GLP-1R agonist such as exendin-4 enhances GSIS and improves glycemic control in diabetic animals and humans, whereas administration of a GLP-1R antagonist (exendin 9–39amide) or genetic disruption of the GLP-1R gene in mice impairs glucose control (4–7). Intriguingly, although GIP receptor (GIPR) agonists potentiate GSIS, GIP antagonists, or genetic ablation of the GIPR gene produces a complex metabolic phenotype reflecting the integrated importance of GIP for the function of both β-cells and adipose tissue (8, 9).

Because GLP-1 and GIP are degraded by the protease DPP-4 (10, 11), several strategies have been developed to prolong their half-life in the circulation to enhance their biological effect. Inhibition of DPP-4 activity produces improvements in glucose control in the setting of experimental and clinical diabetes mellitus (1, 12, 13). Conversely, rats with an inactivating DPP-4

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Abbreviations: DPP-4, Dipeptidyl peptidase-4; EGP, endogenous glucose production; GIP, glucose-dependent insulinotropic peptide; GIPR, GIP receptor; GIR, glucose infusion rate; GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; GSIS, glucose-stimulated insulin secretion; OGTT, oral glucose tolerance test; Rd, rate of disposal (glucose use).

mutation exhibit improved glucose tolerance (14) and mice with deletion of the DPP-4 gene exhibit improved glucose tolerance, increased plasma levels of GLP-1, improved GSIS, and resistance to diet-induced obesity (15, 16).

The dominant mechanism whereby DPP-4 inhibition improves glycemic control is assumed to be the prolongation of the half-life of GLP-1 in the circulation, thereby enhancing its incretin effect (stimulation of glucose dependent insulin secretion). However, the increment in plasma levels of the intact incretin hormones with DPP-4 inhibitor treatment is modest, and antidiabetic therapy with DPP-4 inhibitors has little or no effect on insulin secretion (17). This has led to the speculation that the therapeutic benefit of DPP-4 inhibition cannot be fully explained only by prolongation of the GLP-1 half-life in the systemic circulation and that additional mechanism(s) may contribute to the therapeutic effect of DPP-4 inhibitors (18).

In contrast to the well-established effect of incretins on GSIS, their putative effect on insulin action remains incompletely understood. Whereas several studies failed to demonstrate a significant effect of GLP-1 on insulin action in humans (19–21), Prigeon *et al.* (22) found enhanced suppression of endogenous glucose production by GLP-1 in men under fasting conditions. Similarly, GLP-1 infusion in type 2 diabetic patients subjected to hyperglycemic clamp enhanced glucose disposal, although a trend toward lower glucagon levels may have explained part of this effect (23). Moreover, preclinical studies of the effects of GLP-1 or DPP-4 inhibition on insulin sensitivity have yielded variable results, often confounded by changes in levels of insulin or glucagon, although some studies have shown that GLP-1 increases insulin sensitivity in depancreatized models or the presence of somatostatin infusion, which suppresses insulin and glucagon secretion (13, 24–29).

The aim of the present study, therefore, was to examine the acute effect of the DPP-4 inhibitor des-F-sitagliptin on hepatic and extrahepatic insulin action in awake mice using the hyperinsulinemic euglycemic clamp method and compare this effect with that of the synthetic DPP-4-resistant GLP-1R agonist exendin-4. We found that acute DPP-4 inhibition, but not GLP-1R agonist administration, induces a rapid enhancement of insulin-mediated suppression of endogenous glucose production, independently of its effects on pancreatic function. Because this experiment was performed in fasted and somatostatin-infused mice with very low incretin levels, this effect may be independent of any enhancement of incretin levels and action.

Materials and Methods

Experimental animals

Male wild-type mice on a C57BL/6 genetic background were purchased from Charles River Laboratories (Charles River, Québec, Canada) at the age of 6–7 wk and were housed in the animal facility of the MaRS Centre/Toronto General Research Institute with a 12-h light, 12-h dark cycle for at least 1 wk before the experiment. They received a regular chow diet and water *ad libitum*. All animal experimentation described here was conducted in accord with accepted standards of humane animal care. All procedures were approved by the Animal Care Committee of the University Health Network, University of Toronto.

Assessment of plasma DPP-4 activity

After a 4-h fasting period, groups of 9-wk-old C57BL/6 male mice were dosed with saline or a bolus of des-F-sitagliptin (Merck Research Laboratories, Rahway, NJ) administered by gavage (25 mg/kg body weight of the salt form) or iv (10 mg/kg body weight) via a catheter inserted into the jugular vein 5 d before the experiment, as described below. The DPP-4 inhibitor des-F-sitagliptin is a chemically related analog of sitagliptin that Merck was willing to provide for our studies before sitagliptin received regulatory approval. Its use in preclinical studies has previously been described (30, 31). Blood was collected from the tail vein at regular time intervals, and DPP-4 activity in plasma was determined as previously described (12). Briefly, a 40-μl plasma sample was incubated at 37 C in the presence of Gly-Pro *p*-nitroanilide (substrate; Sigma-Aldrich, St. Louis, MO), and the rate of its conversion into *p*-nitroaniline by DPP-4-mediated enzymatic action was monitored at 0, 15, 30, 45, and 60 min. Yellow color development was monitored at the 405-nm wavelength. Concentration of *p*-nitroaniline produced by DPP-4 action on the substrate was calculated from a standard curve obtained with known concentrations of *p*-nitroaniline. The kinetics of the enzymatic reaction are directly proportional to the slope of the curve calculated by linear regression. DPP-4 activity is expressed as micromoles per liter units of activity per minute of incubation with the substrate.

Hyperinsulinemic euglycemic clamps

Surgical procedure

Three to 5 days before the experiment, mice were anesthetized with a ketamine (100 mg/kg)/xylazine (7.5 mg/kg) cocktail administered ip, and a catheter (Microrenathane, MRE 0.25; Braintree Scientific Inc., Braintree, MA) was inserted into the right jugular vein and tunneled sc to the back (32). Mice were allowed a 3- to 5-d recovery period, and only mice that lost less than 10% of their preoperative weight were used for subsequent analyses.

Hyperinsulinemic euglycemic clamp

After a 5-h fasting period, a hyperinsulinemic, euglycemic clamp was conducted in awake, tail-restrained mice, as previously described (32) and as depicted in Fig. 1. The infusion studies lasted a total of 230 min. After a baseline blood sample was taken from the tail vein, an iv infusion of [3-^{3}H]glucose tracer (Amersham, Piscataway, NJ) was started (10 μCi bolus, 0.05 μCi/min) for a 100-min equilibration (basal) period. During the last 20 min of the basal period, three sequential blood samples were taken at 10-min intervals from the tip of the tail for determination of basal glucose-specific activity, blood glucose concentration and plasma hormones. At time 0, a primed, continuous (60 mU/kg bolus, 4 mU/kg · min) iv infusion of insulin (Humulin R; Eli Lilly, Indianapolis, IN; prepared in BSA 0.5%) was initiated. Somatostatin (3 μg/kg · min; Sigma-Aldrich) was started simultaneously as previously described (33) to suppress pancreatic hormone secretion and avoid any confounding effect of increased insulin secretion or suppression of glucagon secretion on the clamp outcome. The [3-^{3}H]glucose tracer infusion was continued at the same rate (0.05 μCi/min). Concentration of the tracer and insulin infusates was calculated for an infusion rate of 2 μl/min. Blood glucose was measured every 10 min throughout the experiment using a glucometer (in 1 μl blood) and 25% dextrose (containing adequate amount of [3-^{3}H]glucose to maintain glucose specific activity) was infused at a variable rate to maintain plasma glucose at approximately 7 mM. Steady state (achieved within 100–130 min) was when a fixed glucose infusion rate to maintain blood glucose was constant for 30 min. Blood samples were collected at 10-min intervals during steady state (last 30 min of the clamp) for glucose-specific activity and hormone measurement.

Treatment

At time 15 min (*i.e.* 15 min after the start of the insulin and somatostatin infusion), a bolus of saline or des-F-sitagliptin (Merck Research Laboratories; 15 mg/kg body weight of the salt form =11 mg/kg body weight of des-F-sitagliptin) or exendin-4 (50 ng per 25 g body weight)

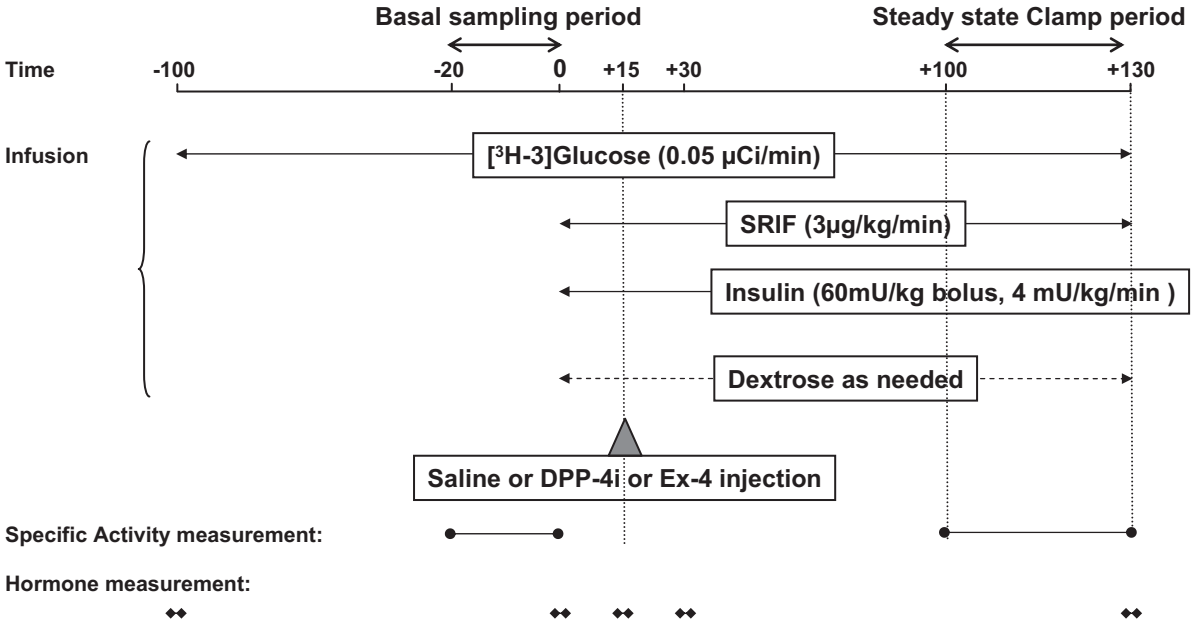

FIG. 1. Schematic representation of the pancreatic and hyperinsulinemic euglycemic clamp procedure. During the first 100 min (equilibration period), only [3-^{3}H]glucose tracer was infused and continued throughout the entire study. Blood samples were collected at time −100 and between times −20 min and 0 min for basal plasma hormone and glucose-specific activity measurements, respectively. At time 0, a primed, continuous (60 mU/kg, 4 mU/kg · min) insulin infusion was started and continued for 130 min. Somatostatin (SRIF) infusion was started at time 0 and was continued for the remainder of the experiment to suppress pancreatic hormone secretion. At time 15 (*i.e.* 15 min after the start of the insulin and somatostatin infusions, saline or exendin-4 (Ex-4; 50 ng per 25 g body weight), or DPP-4 inhibitor (11 mg/kg body weight) was administered as a single 25-μl bolus. Then 25% dextrose (containing adequate amount of [3-^{3}H]glucose to maintain glucose specific activity constant) was infused at a variable rate to maintain plasma glucose at approximately 7 mmol/liter. Steady state (achieved within 100–130 min) was when a fixed glucose infusion rate to maintain blood glucose was constant for 30 min. During that half-hour period, blood samples were collected for the clamp period glucose-specific activity and hormones.

was administered iv. A blood sample was taken 15 min later for hormone measurement.

Oral glucose tolerance test (OGTT)

C57BL/6 male mice were chronically cannulated as described above (these mice were different from those undertaking the hyperinsulinemic euglycenic clamp and OGTT were performed as separate experiments). After 5 d recovery, mice were fasted for 4 h. A baseline blood sample was collected before the iv (through the iv catheter) administration of a 25-μl bolus of either saline (n = 5) or exendin-4 (50 ng/25 g body weight, n = 5). Immediately after the iv administration of saline or exendin-4, mice received a bolus of glucose (1.5 g/kg) by oral gavage (time 0). Blood samples were collected at frequent time intervals from the tail tip for glucose measurement (BD glucometer; Becton-Dickinson, Lincoln Park, NJ). For plasma insulin determinations, a blood sample (50 μl) was removed from the tail vein at 5 min after glucose administration.

Assays

Insulin, C-peptide, and glucagon concentrations were determined by RIA (Linco Research Inc., St. Charles, MO) following the manufacturer's guidelines. For the determination of plasma [3-^{3}H] glucose, plasma was deproteinized with $ZnSO_4$ and $Ba(OH)_2$, dried to remove ^3H_2O, resuspended, and counted in scintillation fluid (Beckman Coulter, Fullerton, CA). GLP-1 plasma levels were measured by with the mouse Lincoplex kit (Linco; no. MENDO-75K) according to the manufacturer's instructions.

Calculations

Rates of basal glucose turnover and whole-body glucose uptake at the end of the basal period and during the final 30 min of the hyperinsulinemic euglycemic clamp were calculated by use of a modified form of Steele's equation, which takes into account the extra tracer infused with the glucose infusate (34). Endogenous glucose production rate (EGP) during clamps was determined by subtracting the glucose infusion rate from the total glucose rate of appearance.

Statistics

All values are presented as mean ± SEM. Differences were considered statistically significant at $P < 0.05$. Comparisons among groups were made using ANOVA followed by pair-wise Scheffé *post hoc* analysis.

Results

Determination of optimal dosing of des-F-sitagliptin and exendin-4

Because doses of up to 576 mg/kg des-F-sitagliptin [1.1% (wt/wt) in food] have been reported by others to result in no toxicity when administered to mice on a daily basis for up to 11 wk (24), we carried out preliminary studies to assess the effects of acute des-F-sitagliptin administration on plasma DPP-4 activity. des-F-sitagliptin (10 mg/kg) administered iv was almost as effective as 25 mg/kg administered orally, and both administration regimens resulted in greater than 80% inhibition of DPP-4 activity for the duration of the 130-min clamp study (Fig. 2).

Exendin-4 has been used at concentrations ranging from 50 to 500 ng per 25 g body weight in mice to study glucose homeostasis (9, 35). Initially we performed hyperinsulinemic, euglycemic clamp studies using a dose of 200 ng exendin-4 (per 25 g body weight) administered as an iv bolus 15 min after starting the insulin infusion. At that concentration of exendin-4 (200 ng per 25 g body weight), the glucose infusion rate (GIR) was signifi-

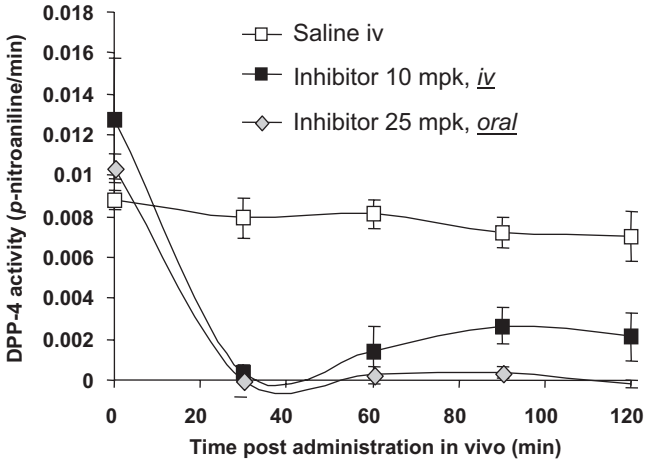

FIG. 2. des-F-sitagliptin effectively inhibits DPP-4 activity. These mice were different from those undertaking the hyperinsulinemic euglycemic clamp. des-F-sitagliptin (DPP-4 inhibitor) was given by either oral gavage (25 mg/kg body weight in 0.5% carboxymethylcellulose, n = 3) or iv (10 mg/kg body weight in saline, jugular vein catheter, n = 3) to 12- to 13-wk C57BL/6 male mice after a 3- to 4-h fasting period. A control group received an iv bolus of saline (n = 3). Blood samples were collected for a 2-h period, and DPP-4 activity was measured as described in *Materials and Methods*.

cantly increased (not shown), but interpretation of this effect was confounded by the simultaneous breakthrough stimulation of insulin secretion as demonstrated by elevated clamp C-peptide plasma levels, despite concurrent somatostatin infusion (Fig. 3A). In contrast, a lower dose of exendin-4, 50 ng per 25 g body weight did not result in breakthrough insulin secretion during the clamp study (Fig. 3A). Moreover, exendin-4 at a concentration of 50 ng per 25 g body weight significantly reduced glucose excursion and increased plasma insulin during an OGTT (Fig. 3, B and C, respectively), demonstrating robust biological activity of this dose of exendin 4.

Acute DPP-4 inhibition but not exendin-4 administration potentiates the effect of insulin *in vivo*

Hyperinsulinemic, euglycemic clamps were performed to assess whether acute DPP-4 inhibition or administration of exendin-4 affects insulin sensitivity *in vivo* using the protocol described above as depicted in Fig. 1. Blood glucose was effectively clamped around 7 mmol/liter and was not significantly different between the two groups (saline, 6.9 ± 0.2 mmol/liter *vs.* exendin-4, 7.2 ± 0.4 mmol/liter *vs.* DPP-4 inhibitor, 7.04 ± 0.2 mmol/liter, $P > 0.05$) (Fig. 4A). Despite matched plasma glucose concentrations, the GIR required to maintain euglycemia was significantly higher (within 15 min) in mice treated with des-F-sitagliptin compared with saline or exendin-4 and remained higher during the remaining clamp period (mean GIR time 100–130 min: des-F-sitagliptin, 60.5 ± 3.3 mg/kg · min *vs.* saline, 42.0 ± 2.7 mg/kg · min, $P < 0.001$, or exendin-4, 46.5 ± 2.9 mg/kg · min, $P < 0.001$; exendin-4 *vs.* saline, P = ns) (Fig. 4B). This effect was not due to an increase in insulin secretion during DPP-4 inhibition, as demonstrated by the absence of differences in plasma insulin levels in saline *vs.* des-F-sitagliptin-treated animals (Fig. 4C), permitting a valid comparison of the relative insulin-potentiating effects of des-F-sitagliptin *vs.* saline. Specific activity during the clamp is shown in Fig. S1 published as supplemental data on The Endocrine Society's Journals Online web site at http:// endo.endojournals.org. In addition, plasma C-peptide levels were appropriately suppressed during the clamp in both experimental groups and were not significantly different between the two groups at t+15 or t+30 min or at the end of the clamp (T+130 min) (Fig. 4D), providing further confirmation that there was no breakthrough of endogenous insulin secretion throughout the clamp period. GLP-1 plasma levels were below the detection limit and therefore cannot be accurately reported. These data indicate that des-F-sitagliptin induced a rapid insulin potentiation independent of any effect on insulin secretion.

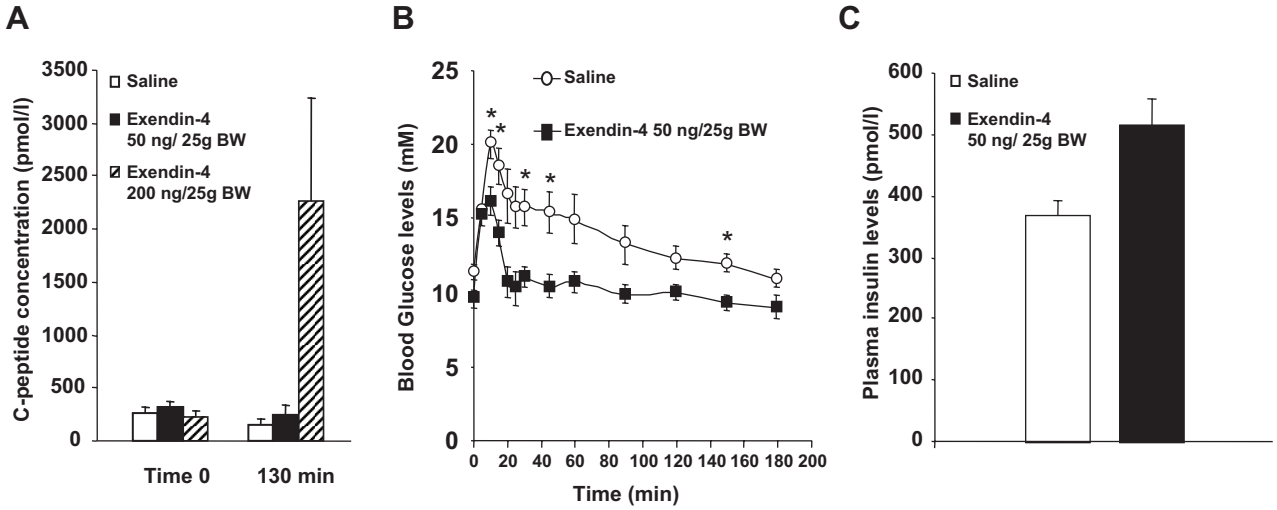

FIG. 3. An iv bolus of 50 ng per 25 g body weight (BW) exendin-4 has an incretin effect *in vivo* and does not cause induction of insulin secretion during the clamp experiments These mice were different from those undertaking the hyperinsulinemic euglycemic clamp experiments reported in the manuscript, and OGTTs were performed as separate experiments. A, Plasma C-peptide levels at baseline and at the end of the hyperinsulinemic euglycemic clamp. Fifty or 200 ng per 25 g body weight exendin-4 was given as a single bolus at time +15 min. B, Blood glucose during an OGTT performed using mice administered 50 ng per 25 g body weight exendin-4 (n = 3) or saline (n = 3), performed as described in *Materials and Methods*. C, Insulin levels in plasma 10 min after injection of exendin-4 (50 ng per 25 g body weight, n = 3) or saline (n = 3). *, $P < 0.05$ by unpaired *t* test.

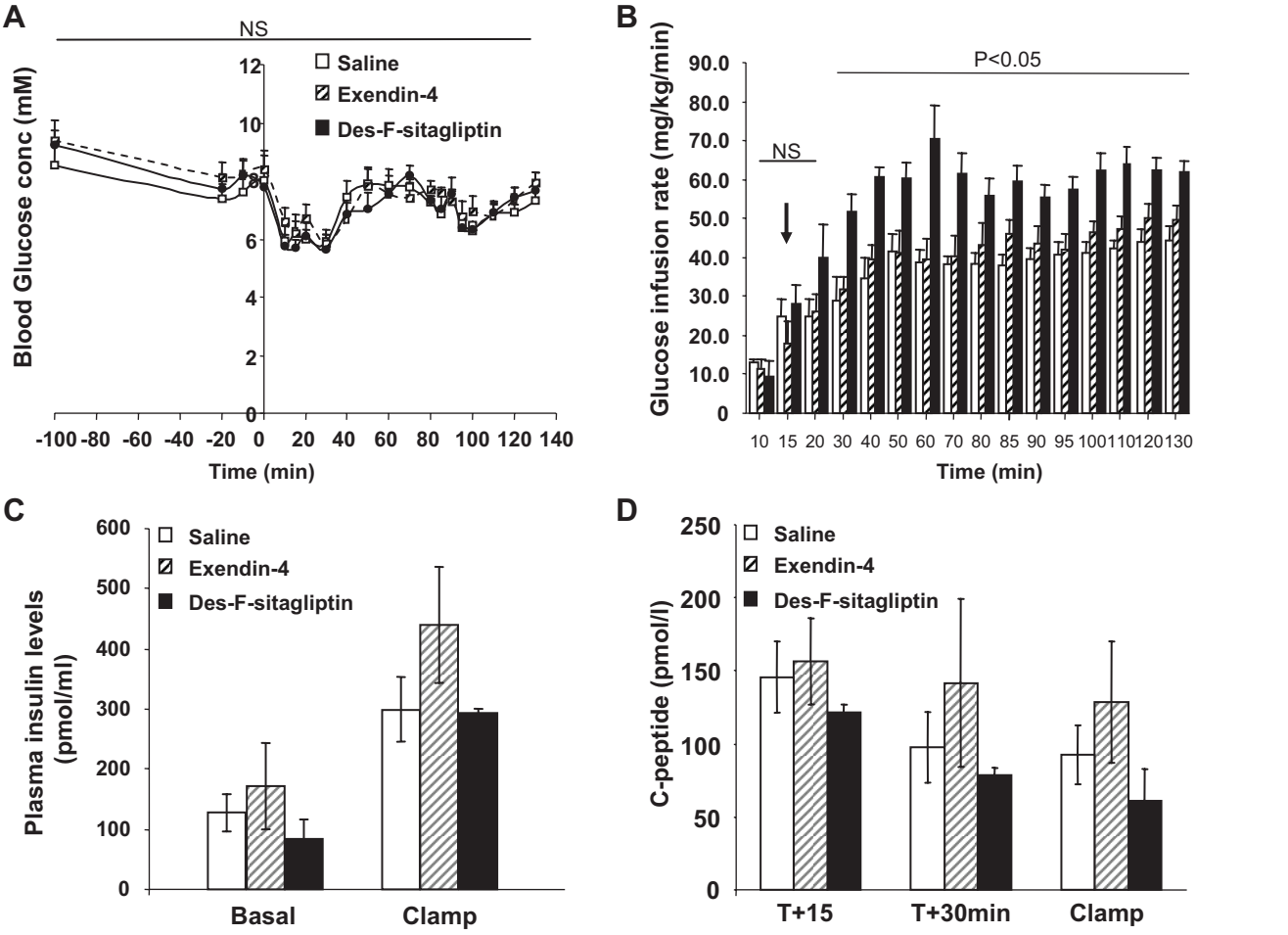

FIG. 4. A rapid increase in GIR is required to maintain euglycemia after DPP-IV inhibition *vs.* exendin-4 and saline. Blood glucose (A), GIR (B), plasma insulin (C) and C-peptide (D) during the hyperinsulinemic euglycemic clamp in mice received a bolus of saline (n = 5), exendin-4 (50 ng/25 g body weight; n = 6), or des-F-sitagliptin (11 mg/kg body weight; n = 6) iv.

Acute DPP-4 inhibition rapidly enhances insulin-mediated suppression of endogenous glucose production

The effect of acute inhibition of DPP-4 on the GIR may reflect either an increased rate of glucose use, greater suppression of EGP (mainly hepatic), or both. [3-^{3}H]glucose tracer analysis demonstrated that this profound difference in insulin action was entirely due to enhancement of insulin-mediated suppression of EGP by des-F-sitagliptin (percent suppression of EGP in des-F-sitagliptin: 121 ± 13%; saline: 38 ± 15%; exendin-4: 19 ± 14%, $P < 0.001$ for des-F-sitagliptin *vs.* saline and exendin-4, P = ns for exendin-4 *vs.* saline) (Fig. 5A). In contrast, insulin-stimulated glucose use (Rd) was not different between the groups (Fig. 5B). In addition, the decrease in EGP after DPP-4 inhibition was not related to changes in glucagon secretion because glucagon levels were not significantly different between saline- and DPP-4-treated mice (basal: saline, 24.76 ± 1.79 *vs.* DPP-4 inhibitor, 35.78 ± 4.67, $P = 0.07$; clamp: saline, 11.85 ± 2.5 *vs.* DPP-4 inhibitor, 8.9 ± 0.78, $P = 0.344$).

Discussion

In the present report, we examined the effect of acute des-F-sitagliptin administration on whole-body glucose metabolism in wild-type, chow-fed mice, independent of its effect on insulin secretion and compared this effect to that of a GLP-1R agonist. We clamped plasma glucose levels in the high euglycemic range (7 mmol/liter) and used a rather low insulin infusion rate (4 mU/kg · min) to compare the various treatments on suppression

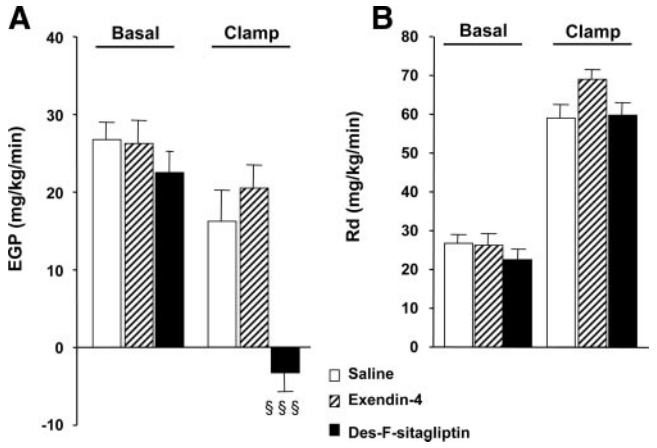

FIG. 5. The profound difference in insulin sensitivity by des-F-sitagliptin is due to enhancement of insulin-mediated suppression of EGP, with no effect on Rd. EGP (A) and Rd (B) during (pancreatic) hyperinsulinemic euglycemic clamp in mice receiving a bolus of saline (n = 5), exendin-4 (50 ng; n = 6) or des-F-sitagliptin (11 mg/kg; n = 6) iv is shown. §§§, $P < 0.001$.

of EGP. Our data clearly show that acute inhibition of DPP-4 activity after des-F-sitagliptin administration completely suppresses EGP (mainly hepatic), with no significant effect on the rate of glucose disposal. Moreover, we used a simultaneous infusion of somatostatin to ensure that insulin and glucagon concentrations did not differ between study groups. The greater suppression of EGP with des-F-sitagliptin therefore was not simply due to greater suppression of glucagon secretion nor was it attributable to greater stimulation of endogenous insulin secretion.

A single dose of the DPP-4 inhibitor vildagliptin in diabetic humans effectively suppressed hepatic glucose output with no change in glucose use in a meal tolerance test (36), an effect likely related to increased insulin and decreased glucagon levels. In contrast, vildagliptin administration has also been shown to induce insulin sensitivity with no effect on EGP through increased Rd (37). It is important to note that our study examined the effect of acute DPP-4 inhibition, whereas chronic DPP-4 inhibition in diabetic rats improved hepatic and peripheral insulin sensitivity as shown by increased GIR, reduced basal and clamp glucose output, and increased Rd at matched insulin concentrations (13). The concomitant reduction in glucolipotoxicity may also contribute to enhanced insulin action in chronic studies, a confounding variable not present in our acute administration paradigm.

Elucidation of the mechanisms important for the glucoregulatory actions of DPP-4 inhibitors have focused on the two dominant incretins, GLP-1 and GIP. Interestingly, GLP-1 infusion in depancreatized dogs was shown to increase GIR and Rd, with no net effect on EGP, in clamp experiments at high insulin concentrations, although the effect of GLP-1 on glucose use was lost at lower insulin doses (25). Studies in mice have demonstrated that the acute or chronic glucoregulatory actions of DPP-4 inhibitors are mediated through the GLP-1 and GIPRs (9, 12). Unexpectedly however, recent studies have demonstrated that $GLP1R^{-/-}$, $GIPR^{-/-}$, and double-incretin receptor knockout mice exhibit complex phenotypes characterized by increased locomotor activity, enhanced energy expenditure, and in some experimental circumstances, enhanced insulin sensitivity (38, 39).

In the present study, des-F-sitagliptin administration had a profound effect on insulin-mediated suppression of EGP, whereas administration of a bolus of exendin-4 did not induce any change to either Rd or EGP. These results indicate that, in our experimental setting, the effect of des-F-sitagliptin on insulin-mediated EGP suppression is unlikely to be mediated by GLP-1. In support of this hypothesis, GLP-1 levels were very low in our experimental conditions, probably as a result both of the somatostatin infusion (somatostatin has been previously shown to suppress GLP-1 as well as GIP both *in vivo* and *in vitro* (40–43) and the 5-h fasting period before the start of the clamp (a total of almost 7 h without food ingestion because the clamp was preceded by a 100 min tracer equilibration period), and DPP-4 inhibition in the fasted state is not expected to lead to a relevant increase of GLP-1 levels. This dissociation between the effect of DPP-4 inhibition by des-F-sitagliptin and GLP-1R activation is consistent with the absence of effect of exendin-4 administration on EGP in the same experimental setting.

Another possible explanation for our observation might involve the action of another incretin, GIP. However, as mentioned above, somatostatin also suppresses GIP secretion, making it unlikely that GIP is involved in the effect of des-F-sitagliptin on EGP. The underlying mechanism may be related in some way to the DPP-4 enzyme's ability to cleave other substrates such as neuropeptides and chemokines (44), resulting in elevated plasma and/or tissue levels of these factors. Hence, future studies are needed to explore how DPP-4 inhibitors rapidly enhance insulin-mediated glucose production suppression and will need to carefully consider the optimal experimental model in light of these observations.

In conclusion, we have demonstrated that acute administration of the DPP-4 inhibitor des-F-sitagliptin induces insulin-mediated suppression of endogenous glucose production. This finding adds to the growing body of knowledge regarding the complex mechanism of action of these antidiabetic agents and serves to emphasize that mechanisms other than incretin-mediated enhancement of GSIS may be important in mediating the glucose-lowering effect of these therapeutic agents.

Acknowledgments

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