Chronic Exposure to GLP-1R Agonists Promotes Homologous GLP-1 Receptor Desensitization In Vitro but Does Not Attenuate GLP-1R–Dependent Glucose Homeostasis In Vivo

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Glucagon-like peptide-1 (GLP-1) stimulates glucose-dependent insulin secretion and inhibits food intake, gastric emptying, and glucagon secretion, actions that promote reduction of fasting and postprandial glycemia in subjects with type 2 diabetes. The rapid degradation of native GLP-1 has engendered interest in more stable longer-acting GLP-1 receptor agonists such as exendin-4 (Ex-4); however, the potential consequences of sustained GLP-1 receptor activation leading to receptor desensitization has not been extensively studied. We have now examined a range of GLP-1 receptor–dependent responses following treatment with Ex-4 using INS-1 cells in vitro and both wild-type control and MT–Ex-4 transgenic mice in vivo. Although both GLP-1 and Ex-4 acutely desensitized GLP-1 receptor–dependent cAMP accumulation in INS-1 cells, Ex-4 produced more sustained receptor desensitization, relative to GLP-1, in both acute (5–120 min) and chronic (24–72 h) experiments. PMA (4-phorbol 12-myristate 13-acetate) but not glucagon, glucose-dependent insulinotropic polypeptide (GIP), or epinephrine produced heterologous desensitization in vitro. MT–Ex-4 transgenic mice exhibited a reduced glycemic response to oral but not intraperitoneal glucose challenge following acute Ex-4 administration. In contrast, no differences in glycemic excursion or plasma insulin were observed after 1 week of twice-daily Ex-4 administration to wild-type versus MT–Ex-4 mice. Similarly, the levels of insulin, pdx-1, and GLP-1 receptor mRNA transcripts were comparable in wild-type and MT–Ex-4 transgenic mice after 1 week of Ex-4 administration. However, repeated Ex-4 administration significantly reduced food intake in MT–Ex-4 but not in wild-type mice. These findings illustrate that although Ex-4 is more potent than native GLP-1 in producing GLP-1 receptor desensitization in vitro, chronic exposure to Ex-4 in normal or transgenic mice is not associated with significant downregulation of GLP-1 receptor–dependent responses coupled to glucose homeostasis in vivo. *Diabetes* 53 (Suppl. 3):S205–S214, 2004

Glucagon-like peptide-1 (GLP-1), a 30–amino acid peptide, is secreted from gut endocrine L-cells in a nutrient-dependent manner (1,2). GLP-1 enhances glucose-dependent insulin secretion and inhibits food intake, gastric emptying, and glucagon secretion, complementary actions that lower blood glucose in both normal subjects and in patients with type 2 diabetes. More recent studies have demonstrated that chronic GLP-1 administration expands islet mass via stimulation of β-cell proliferation and islet neogenesis and inhibition of β-cell apoptosis (3–5). Taken together, these actions have fostered considerable interest in the use of GLP-1R agonists for the treatment of type 2 diabetes.

Acute infusion of GLP-1 is associated with rapid lowering of blood glucose in both normal and diabetic subjects, and a more prolonged period of GLP-1 administration, for 24 h over a 7-day treatment period, produced a greater reduction in nocturnal and fasting glycemia than that obtained with a 16-h treatment protocol (6). Furthermore, chronic administration of native GLP-1 via repeated subcutaneous injection or continuous infusion reduces fasting and postprandial blood glucose and decreases HbA$_{1c}$, in association with modest but significant weight loss (7,8). These findings demonstrate the potential utility of native GLP-1 for the treatment of type 2 diabetes. Nevertheless, progress toward the development of GLP-1–based therapeutic agents has been slow, partly because of the rapid degradation and clearance of the native GLP-1 peptide.

GLP-1 contains an alanine at position 2 and is an excellent substrate for NH$_2$-terminal inactivation by the enzyme dipeptidyl peptidase IV (9,10). Furthermore, GLP-1 is rapidly cleared from the circulation via renal mechanisms that depend in part on neutral endopeptidase 24.11. Hence, levels of circulating intact GLP-1 fall rapidly after subcutaneous injection (11), posing considerable challenges for therapeutic programs using the native unmodified peptide. These findings have prompted the development of stable degradation-resistant GLP-1 analogs that

exert longer durations of action and more sustained pharmacokinetic profiles following parenteral administration.

Current pharmaceutical approaches are evaluating the effectiveness of GLP-1R agonists, such as liraglutide (NN2211) or exenatide (exendin-4 [Ex-4]), for once or twice-daily administration regimens, respectively (12,13). Despite sustained elevations in the circulating levels of these GLP-1R agonists, no evidence for tachyphylaxis or clinically significant receptor desensitization has been observed in clinical studies. Furthermore, there remains interest in developing even longer-acting GLP-1R agonists (3) that might theoretically achieve higher levels of circulating agonists and therefore require administration only once a week. Although short-term exposure to GLP-1 produces rapid receptor desensitization in vitro, there is little information about the potential for more stable long-acting GLP-1R agonists to produce receptor desensitization in vivo. Accordingly, we examined GLP-1R–dependent responses before and after repeated exposure to GLP-1 or Ex-4 using islet cells in vitro and both normal and transgenic mice in vivo.

RESEARCH DESIGN AND METHODS

Cell culture. INS-1 islet cells were cultured in RPMI 1640 medium containing 11 mmol/l glucose and supplemented with 10% (vol/vol) heat-inactivated FCS, 25 mmol/l HEPES [N-(2-hydroxyethyl)piperazine-N-(2-ethanesulphonic acid)], 100 IU/ml penicillin, 100 mg/ml streptomycin, 1 mmol/l sodium pyruvate, and 50 mmol/l mercaptoethanol in a humidified (95% air/5% CO_2) atmosphere. Tissue culture medium was changed every 24 h, and for desensitization studies, 100 nmol/l of fresh GLP-1 or Ex-4 (California Peptide Research, Napa, CA) was added daily. For experiments examining heterologous desensitiza-

tion, 100 nmol/l glucose-dependent insulinotropic polypeptide (GIP), glucagon, epinephrine (Sigma, St. Louis, MO), or 400 nmol/l 4-phorbol 12-myristate 13-acetate (PMA) (Sigma) were added to INS-1 cells for 1- or 24-h periods.
cAMP analyses. INS-1 cells were washed with PBS, incubated for 1 h with fresh RPMI media, then incubated with specific peptides for 30 min in the presence of 100 mmol/l isobutylmethyl xanthine (IBMX) in serum-free medium. Cells were then washed, 70% ethanol supplemented with 100 mmol/l IBMX was added, and extracts were stored at $-20°C$. Analysis of cAMP was via radioimmunoassay (Biomedical Technologies, Stoughton, MA). All experimental treatments were performed in triplicate or quadruplicate.
Mice. The generation and characterization of MT–Ex-4 transgenic mice on a C57BL/6 $\times$ SJL genetic background has been described (14). These mice express Ex-4 in multiple tissues, and plasma levels of Ex-4 generally ranged from 5 to 20 pg/ml. MT–Ex-4 mice and age-matched (12- to 16-week-old males) control (transgene-negative) C57BL/6 mice were maintained on a 12-h light/dark cycle with free access to standard rodent chow and water, except where noted. Experiments were carried out following protocols and guidelines approved by the Toronto General Hospital Animal Care Committee.
Glucose tolerance and plasma insulin measurements. Glucose tolerance was analyzed in control and MT-exendin transgenic mice before (basal) and following 1 week of twice-daily intraperitoneal Ex-4 (100 ng/mouse) or PBS administration. Oral glucose tolerance tests (OGTTs) or intraperitoneal glucose tolerance tests (IPGTTs) were carried out following an overnight fast (16–18 h). Fasting blood glucose was measured and Ex-4 (100 ng/mouse; California Peptide Research) or PBS was administered by intraperitoneal injection immediately before glucose loading. Glucose (1.5 mg/g body wt) was administered via a gavage tube or by intraperitoneal injection. Blood glucose levels were measured using a Glucometer Elite blood glucose meter (Bayer, Toronto, ON). For plasma insulin determination, a blood sample (100 μl) was removed from the tail vein during the 10- to 20-min time period after glucose administration and mixed immediately with a 10% vol of a chilled solution containing 5,000 KIU/ml Trasylol (Bayer), 32 mmol/l EDTA, and 0.1 nmol/l Diprotin A (Sigma). Plasma was stored at $-80°C$. Plasma insulin levels were measured using a rat/mouse insulin enzyme-linked immunosorbent assay kit (Linco Research, St. Charles, MO) with rat insulin as standard.
Pancreas insulin extraction and measurement. The pancreas was homogenized twice in ice-cold extraction medium [1N HCl containing 5% (vol/vol)
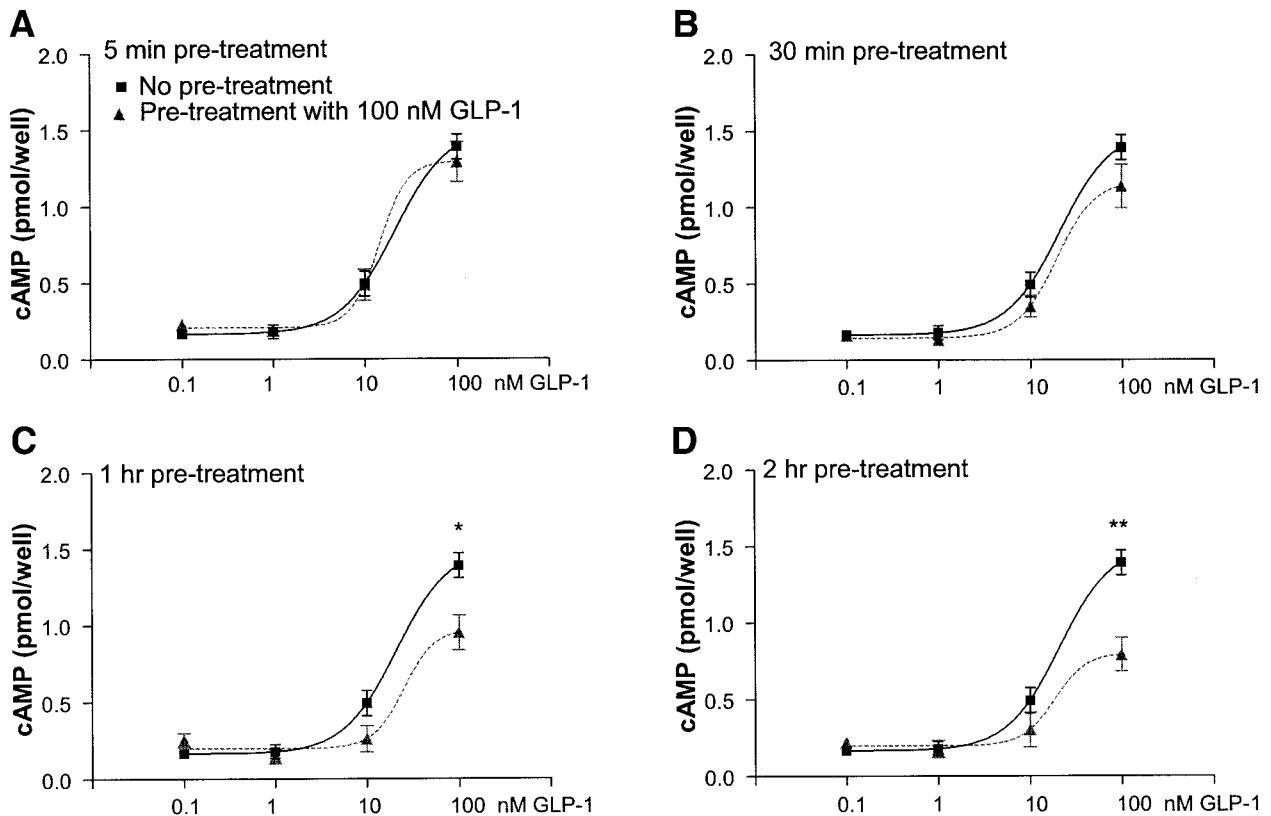
FIG. 1. Short-term homologous desensitization of GLP-1–stimulated cAMP production in INS-1 cells. Cells were pretreated with or without 100 nmol/l GLP-1 for indicated times (5 min to 2 h), followed by a 1-h resensitization period and subsequent stimulation with 0.1, 1, 10, and 100 nmol/l GLP-1 for 30 min. Data are means ± SE of at least four independent experiments. *$P < 0.05$, **$P < 0.01$.
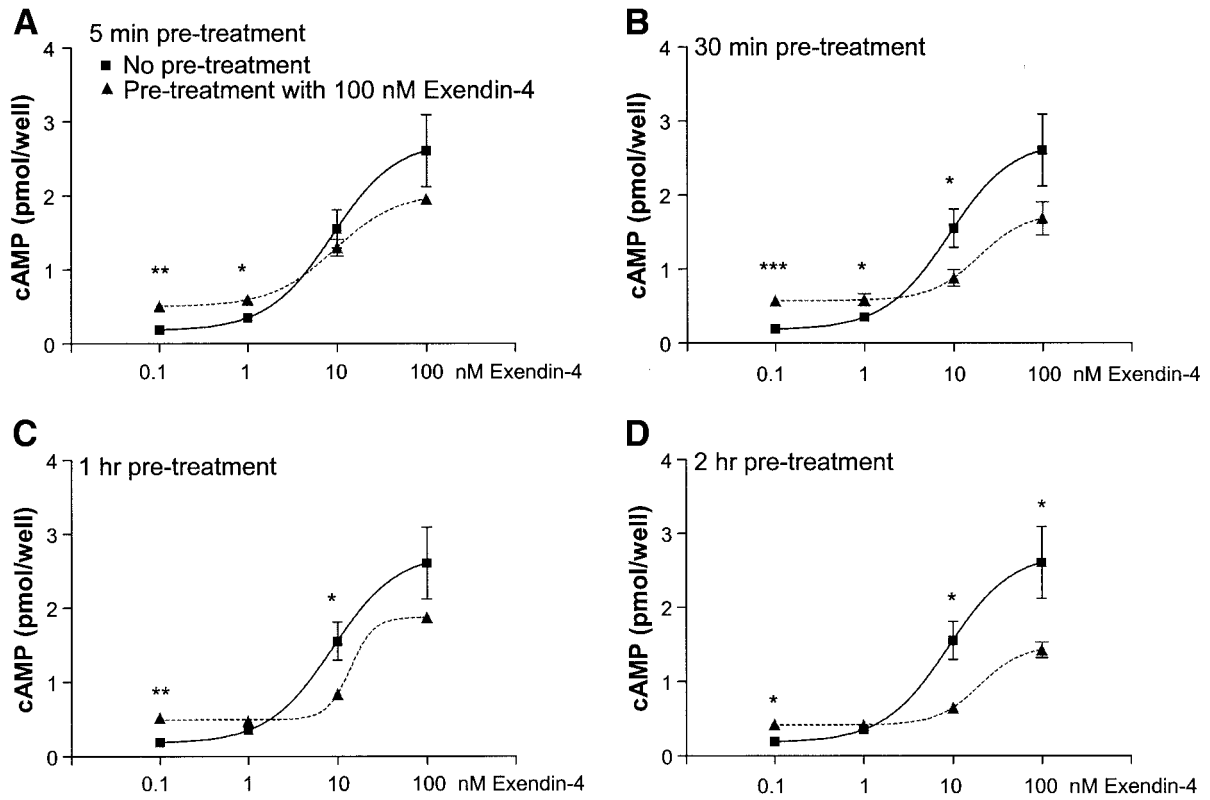
FIG. 2. Short-term desensitization of Ex-4–stimulated cAMP production in INS-1 cells. Cells were pretreated with or without 100 nmol/l Ex-4 for indicated times (5 min to 2 h), followed by a 1-h resensitization period and subsequent stimulation with 0.1, 1, 10, and 100 nmol/l Ex-4 for 30 min. Data are means ± SE of at least four independent experiments. *$P < 0.05$, **$P < 0.01$, *** $P < 0.001$.
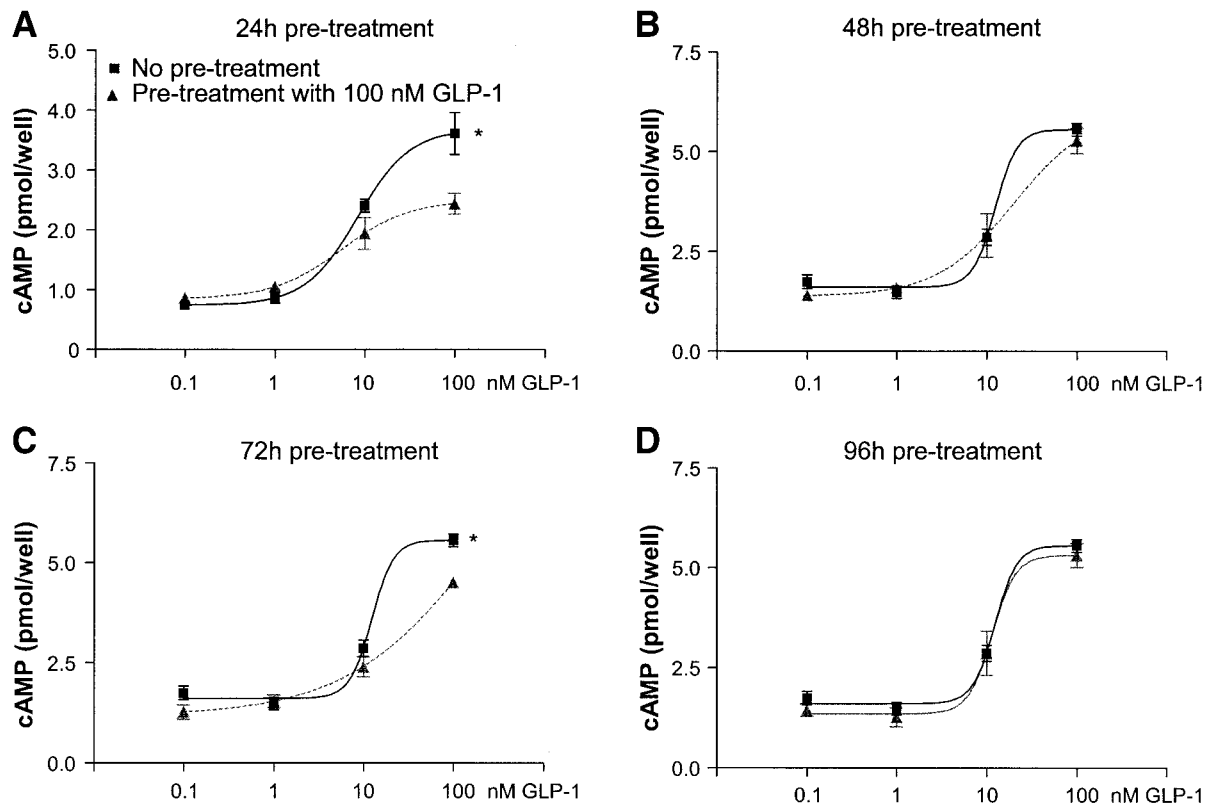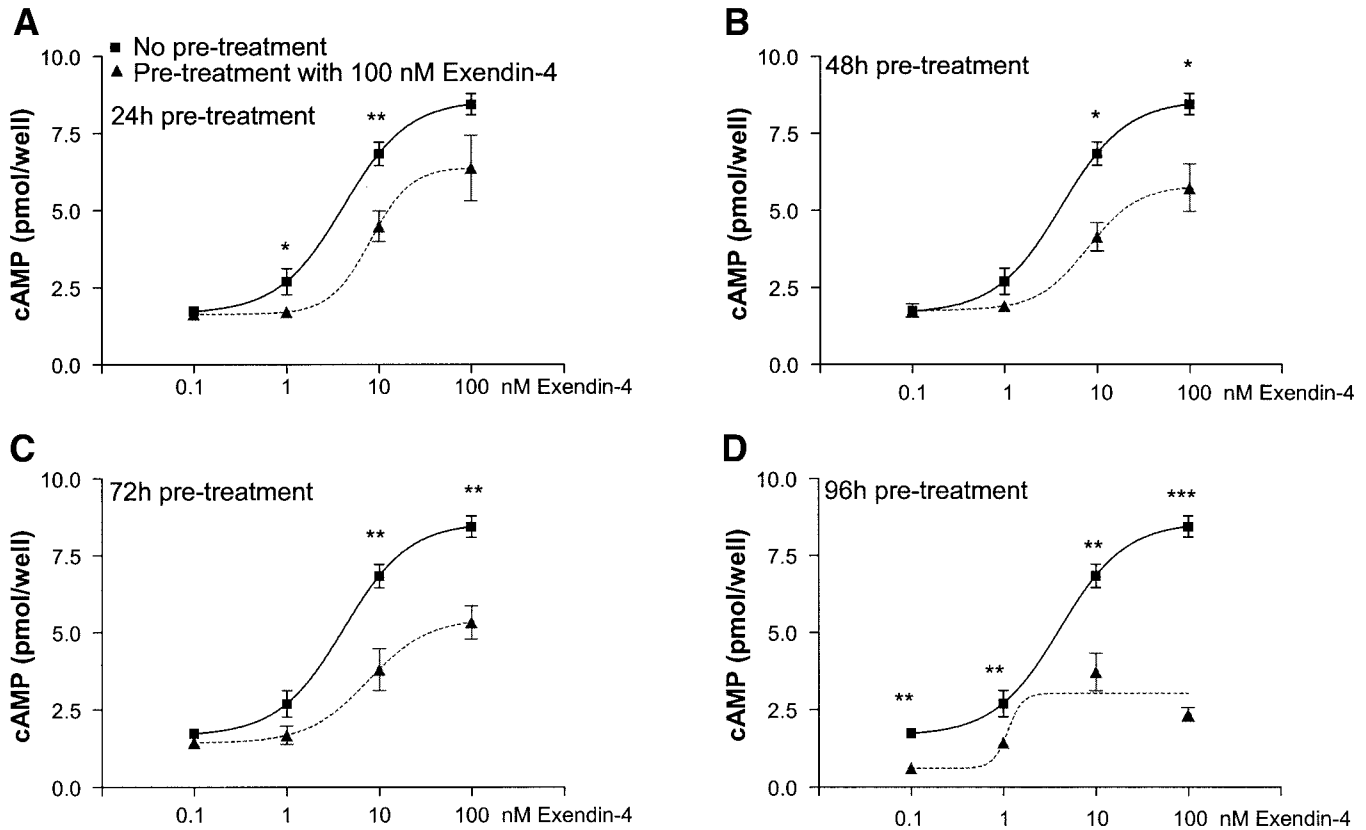

FIG. 3. Prolonged exposure to GLP-1 promotes homologous desensitization of cAMP production in INS-1 cells. Cells were pretreated with or without 100 nmol/l GLP-1 for indicated times (24–96 h), followed by a 1-h resensitization period without peptide and subsequent restimulation with 0.1, 1, 10, and 100 nmol/l GLP-1 for 30 min. *$P < 0.05$.

FIG. 4. Prolonged exposure to Ex-4 promotes desensitization of cAMP production in INS-1 cells. Cells were pretreated with or without 100 nmol/l Ex-4 for indicated times (24–96 h), followed by a 1-h resensitization period and resubsequent stimulation with 0.1, 1, 10, and 100 nmol/l Ex-4 for 30 min. Data are means ± SE of at least four independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

formic acid, 1% (vol/vol) trifluoroacetic acid, and 1% (wt/vol) NaCl]. Peptides and small proteins were adsorbed from extracts by passage through a C18 silica cartridge (Waters Associates, Milford, MA). Adsorbed peptides were eluted in 80% (vol/vol) isopropanol containing 1% (vol/vol) trifluoroacetic acid. Pancreatic insulin was measured using a rat insulin radioimmunoassay kit (Linco Research) with rat insulin as a standard. Total protein concentration in pancreatic extracts was measured using the Bradford method (15) with dye reagent (Bio-Rad Laboratories, Hercules, CA).

RNA isolation and real-time RT-PCR. RNA was extracted from pancreas samples using TRI reagent (Sigma). Real-time quantitative PCR reactions were carried out using an Applied Biosystems 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Primers and PCR conditions for mouse insulin, pdx-1, and the GLP-1R transcripts were those recommended by Applied Biosystems.

Feeding studies. Mice were weighed, given intraperitoneal injections of Ex-4 (100 ng/mouse), and then placed into individual cages containing preweighed rodent diet, with free access to water. At 2, 4, 8, and 24 h following Ex-4 administration, the chow was reweighed and total food intake (g/g body wt) was calculated.

Statistics. For analysis of cAMP, data were plotted using the nonlinear regression method and statistical significance was assessed with an unpaired Student's t test.

Data for analysis of wild-type and MT-exendin mice are presented as means ± SE. Statistical significance was determined by Student's t test or ANOVA and Bonferonni's post-test using Prism Version 3.03 software (Graph-Pad Software, San Diego, CA). A P value <0.05 was considered to be statistically significant.

RESULTS

Studies utilizing short-term incubations (5–30 min) with GLP-1 in islet cell lines have demonstrated desensitization of the GLP-1 receptor in vitro (16). To compare the effects of native GLP-1 versus the more stable degradation-resistant lizard peptide Ex-4 on GLP-1 receptor desensitization, we preincubated INS-1 islet cells with GLP-1 or Ex-4 from

5 min to 2 h, following which cells were rechallenged with the same peptide. No significant diminution in the magnitude of GLP-1–stimulated cAMP accumulation was seen following preincubation of INS-1 cells with 100 nmol/l GLP-1 for 5 or 30 min (Fig. 1); however, longer preincubation periods for 1–2 h produced a significant reduction in the cAMP response following subsequent rechallenge with GLP-1 (Fig. 1*A*–*D*). In contrast, INS-1 cells incubated with Ex-4 for 5 or 30 min exhibited higher basal levels of cAMP (Fig. 2*A* and *B*). Furthermore, a significantly diminished magnitude of cAMP accumulation following Ex-4 rechallenge was detected in cells preincubated with Ex-4 for as little as 30 min (Fig. 2*B*).

The majority of previous studies examining GLP-1 receptor desensitization in islet cells have used short (5–60 min) preincubation periods; however, little information is available about the consequences of long-term exposure of islet cells to GLP-1 receptor agonists. Accordingly, we assessed the cAMP response to GLP-1 or Ex-4 following 24- to 96-h pretreatment periods. Evidence for a diminished cAMP response was detected in INS-1 cells pretreated with 100 nmol/l GLP-1 for 24 or 72 h, but was only detected when cells were rechallenged with 100 nmol/l GLP-1 (Fig. 3*A* and *C*). In contrast, significantly reduced cAMP accumulation following rechallenge with Ex-4 was observed in cells preincubated with 100 nmol/l Ex-4 for 24–96 h (Fig. 4*A*–*D*), and significantly reduced cAMP accumulation was observed even at concentrations as low as 0.1 nmol/l Ex-4 after a 96-h pretreatment period (Fig. 4*D*).
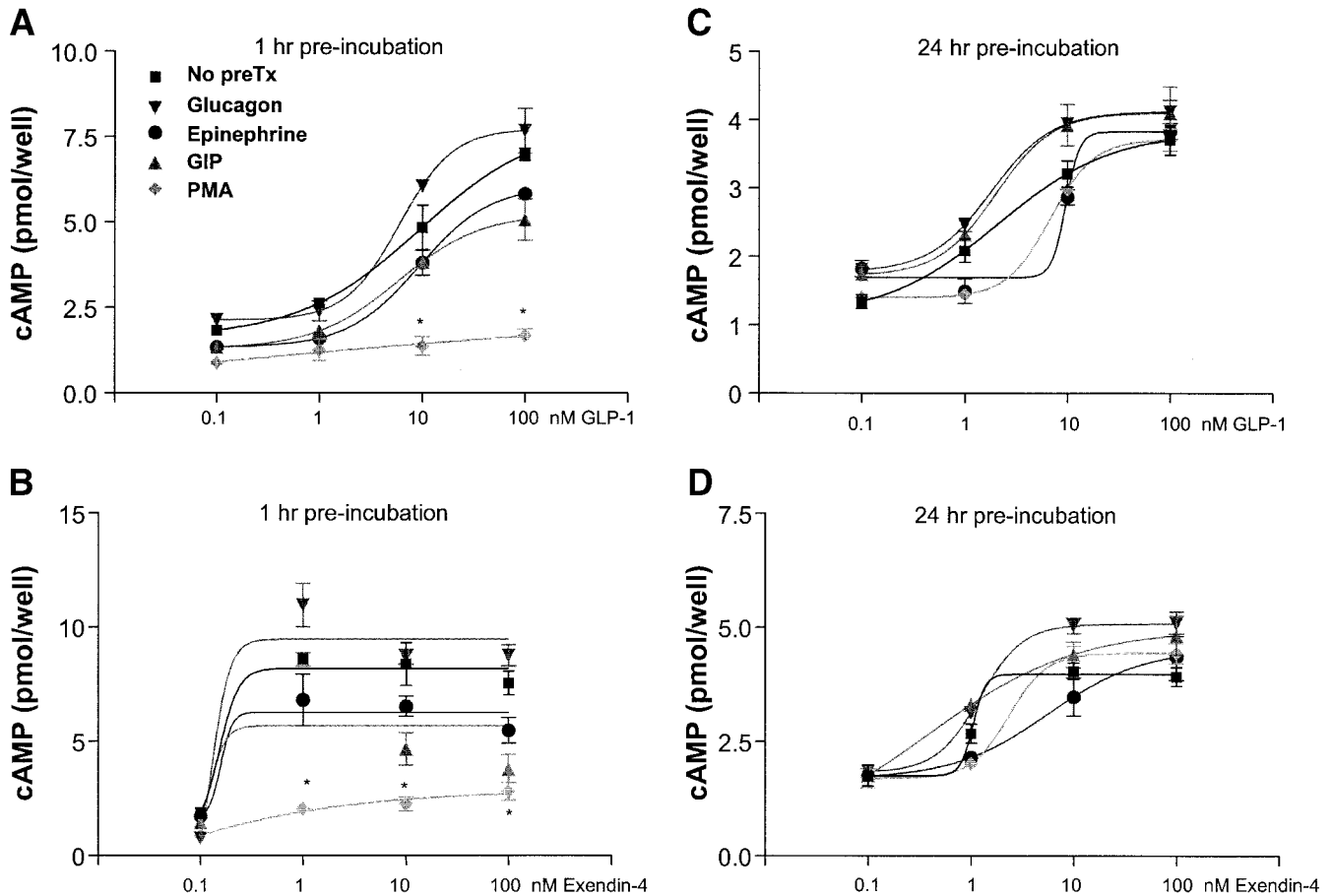
FIG. 5. Heterologous desensitization of GLP-1–stimulated (*A* and *C*) or Ex-4–stimulated (*B* and *D*) cAMP production in INS-1 cells. Cells were pretreated with 100 nmol/l glucagon, epinephrine, or GIP or 400 nmol/l PMA for 1 h (*A* and *B*) or 24 h (*C* and *D*), followed by a 1-h resensitization period and subsequent restimulation with 0.1, 1, 10, and 100 nmol/l GLP-1 (*A* and *C*) or Ex-4 (*B* and *D*) for 30 min. *$P < 0.05$.

The results of these homologous desensitization experiments demonstrated significant differences in the INS-1 cell response to preincubation and rechallenge with native GLP-1 versus Ex-4. To determine if INS-1 cells exhibited differential responsivity to these ligands following preincubation with heterologous agents that also activate the secretory pathway, we examined the cAMP response to GLP-1 or Ex-4 after preincubation of INS-1 cells with glucagon, GIP, epinephrine, or PMA. A 1-h preincubation with PMA was associated with significant diminution in the magnitude of GLP-1 or Ex-4–stimulated cAMP accumulation (Fig. 5*A* and *B*). In contrast, a 24-h preincubation with glucagon, GIP, or epinephrine was not associated with heterologous desensitization to subsequent rechallenge with GLP-1 or Ex-4 (Fig. 5*C* and *D*).

To determine whether repeated administration of Ex-4 was associated with evidence for GLP-1 receptor desensitization in vivo, we analyzed glucose homeostasis in wild-type and MT–Ex-4 transgenic mice treated with twice-daily Ex-4 for 7 days. Wild-type mice initially exhibited a greater magnitude (relative to MT–Ex-4 mice) of glycemic excursion following OGTT, and the area under the curve (AUC) glucose was significantly reduced following treatment with Ex-4, both before and after 1 week of twice-daily Ex-4 administration (Fig. 6*A–D*). Although the magnitude of AUC glycemic excursion following oral glucose loading was reduced in MT–Ex-4 relative to control wild-type mice, exogenous Ex-4 reduced the AUC glucose in MT–EX-4 mice both acutely and after 1 week of twice-daily EX-4 administration (Fig. 6*A–D*). Intriguingly, the levels of plasma insulin were significantly lower in saline- or Ex-4–treated MT–Ex-4 mice, relative to control mice, at the end of the 1-week treatment period (Fig. 6*E* and *F*). Intraperitoneal glucose tolerance was assessed in a separate group of control and MT–Ex-4 transgenic mice before and 1 week after twice-daily Ex-4 administration (Fig. 6*G* and *H*). In contrast to the results of the OGTT in wild-type versus MT–Ex-4 mice (Fig. 6*A*), glycemic excursion after IPGTT was identical in control- and MT–Ex-4 transgenic mice treated with a single acute injection of Ex-4 (Fig. 6*G*), and no differences in glucose clearance, relative to the baseline IPGTT, were observed after 1 week of twice-daily Ex-4 administration (Fig. 6*H*).

Body weight and basal food intake were not significantly different before the 1-week treatment period in wild-type versus MT–Ex-4 transgenic mice (Fig. 7*A*); however, 1 week of Ex-4 administration significantly inhibited food intake to a greater extent in MT–Ex-4 transgenic compared with wild-type control mice (Fig. 7*B*). No significant differences in body weight were detected after 1 week of Ex-4 administration in wild-type versus MT–Ex-4 mice (Fig. 7*C*). Although pancreatic weight was comparable in
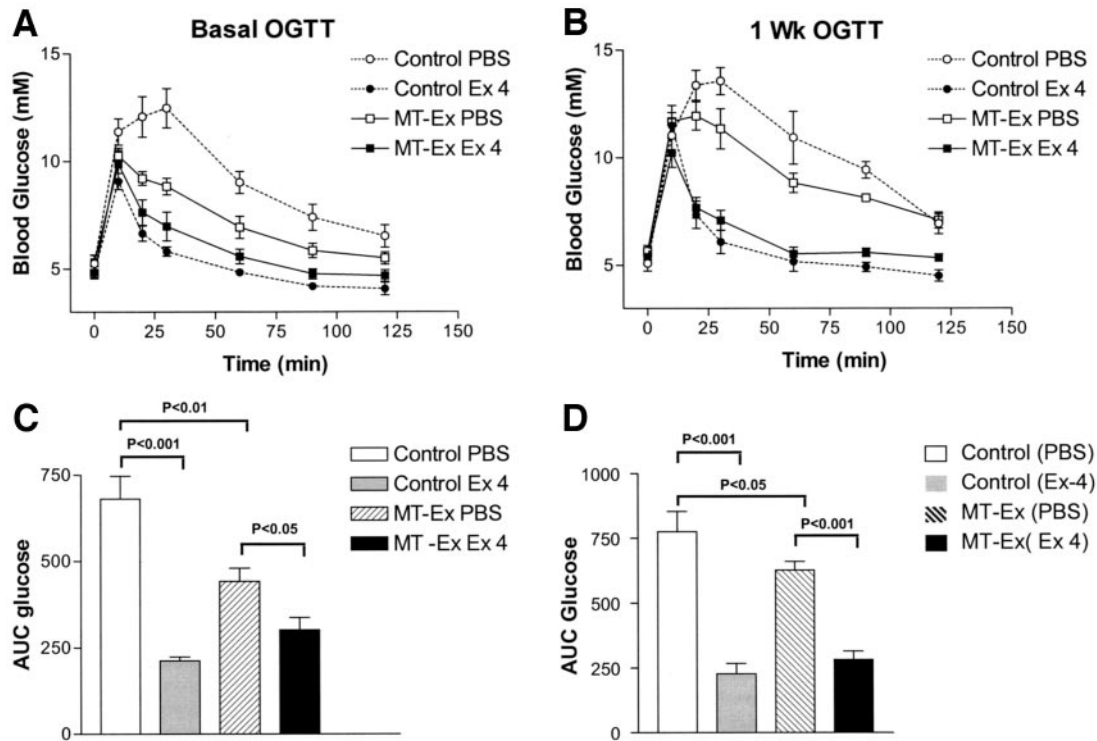
FIG. 6. Improved oral glucose tolerance in control and MT-exendin transgenic mice in response to Ex-4 treatment. Basal OGTT (*A*) and AUC glucose (*C*) in control and MT-exendin transgenic (MT-Ex) mice following intraperitoneal injection of PBS or 100 ng Ex 4. OGTT (*B*) and AUC glucose (*D*) in control and MT-exendin mice following 1 week of twice-daily administration of intraperitoneal PBS or Ex-4 (100 ng). Values are means ± SE; *n* = 4–8 mice/group. Plasma insulin levels at the 10- to 20-min time point following glucose challenge during a basal OGTT before 1 week of Ex-4 administration in control and MT-Ex mice following IP injection of PBS or 100 ng Ex-4 (*E*) or levels of glucose-stimulated insulin during OGTT after 1 week of twice-daily administration of IP PBS or 100 ng Ex-4 (*F*). Values are means ± SE; *n* = 4–8 mice/group. **P* < 0.01 for PBS-treated control mice vs. PBS-treated MT-exendin mice; #*P* < 0.05 for Ex-4–treated control mice vs. Ex-4–treated MT-Ex mice. *G*: Basal IPGTT and AUC glucose in control and MT-exendin mice following intraperitoneal injection of the first injection of Ex-4 (100 ng) at the start of the 1-week treatment period. *H*: IPGTT and AUC glucose in control and MT-exendin mice following 1 week of twice-daily administration of intraperitoneal PBS or Ex-4 (100 ng). Values are means ± SE; *n* = 4–5 mice/group.

wild-type mice after saline versus Ex-4 administration, pancreatic weight was significantly increased in MT–Ex-4 mice following Ex-4 treatment (Fig. 7*D*). In contrast, pancreatic insulin content was significantly increased in wild-type mice, but not in MT–Ex-4 mice, after 1 week of Ex-4 administration (Fig. 7*E*).

To determine the effect of chronic repeated exposure to Ex-4 on pancreatic mRNA transcripts potentially upregulated (insulin, pdx-1) or downregulated (GLP-1 receptor) following GLP-1 receptor activation, we used real-time PCR to determine the levels of these mRNA transcripts at the end of the 1-week treatment period. No significant differences in the levels of pancreatic insulin, pdx-1, and GLP-1 receptor mRNA transcripts were detected in wild-type versus MT–Ex-4 saline-treated mice, and Ex-4 treatment had no significant effect on the levels of these mRNA transcripts in wild-type or transgenic mice at the end of the 7-day treatment period (data not shown).

DISCUSSION

Given the clinical interest in the therapeutic benefits of achieving sustained chronic elevations in plasma levels of GLP-1 receptor agonists, the potential for GLP-1 receptor desensitization has implications for the optimal use of GLP-1 receptor agonists. Receptors for glucagon, GLP-2, and GIP undergo rapid homologous desensitization in vitro (16–18). Similarly, short-term incubations of islet cells with native GLP-1 induces rapid GLP-1 receptor

desensitization (16,19), accompanied by phosphorylation of serine residues in the COOH-terminal tail of the receptor (20,21). Desensitization of the islet GLP-1 receptor was also observed with acute exposure to PMA (19), in agreement with our findings using PMA in the current study. Furthermore, mutation of specific serine residues in the COOH-terminal tail of the GLP-1 receptor results in diminished PMA-associated receptor desensitization (21,22).

Continuous exposure of isolated rat islets to the related incretin GIP for 20 h resulted in a reduction in the subsequent insulin secretory response to either GIP or glucose (23). Similarly, a 4-h infusion of GIP in rats produced an initial but nonsustained increase in plasma insulin, whereas a comparable infusion of GLP-1 maintained elevated levels of plasma insulin during the entire 4-h time period (24). Although exposure of islet cells to GIP produces homologous desensitization of the GIP receptor, exposure of HIT islet cells to GIP had no effect on the subsequent response to GLP-1. In contrast, preexposure of HIT cells to GLP-1 alone produced homologous GLP-1 receptor desensitization (16). These findings are in agreement with our data generated using INS-1 cells, which failed to detect evidence for heterologous desensitization of the GLP-1 receptor after exposure to epinephrine, GIP, or glucagon. Hence, despite overlapping biological actions converging on insulin secretion, GIP and GLP-1 do not seem to produce meaningful heterologous
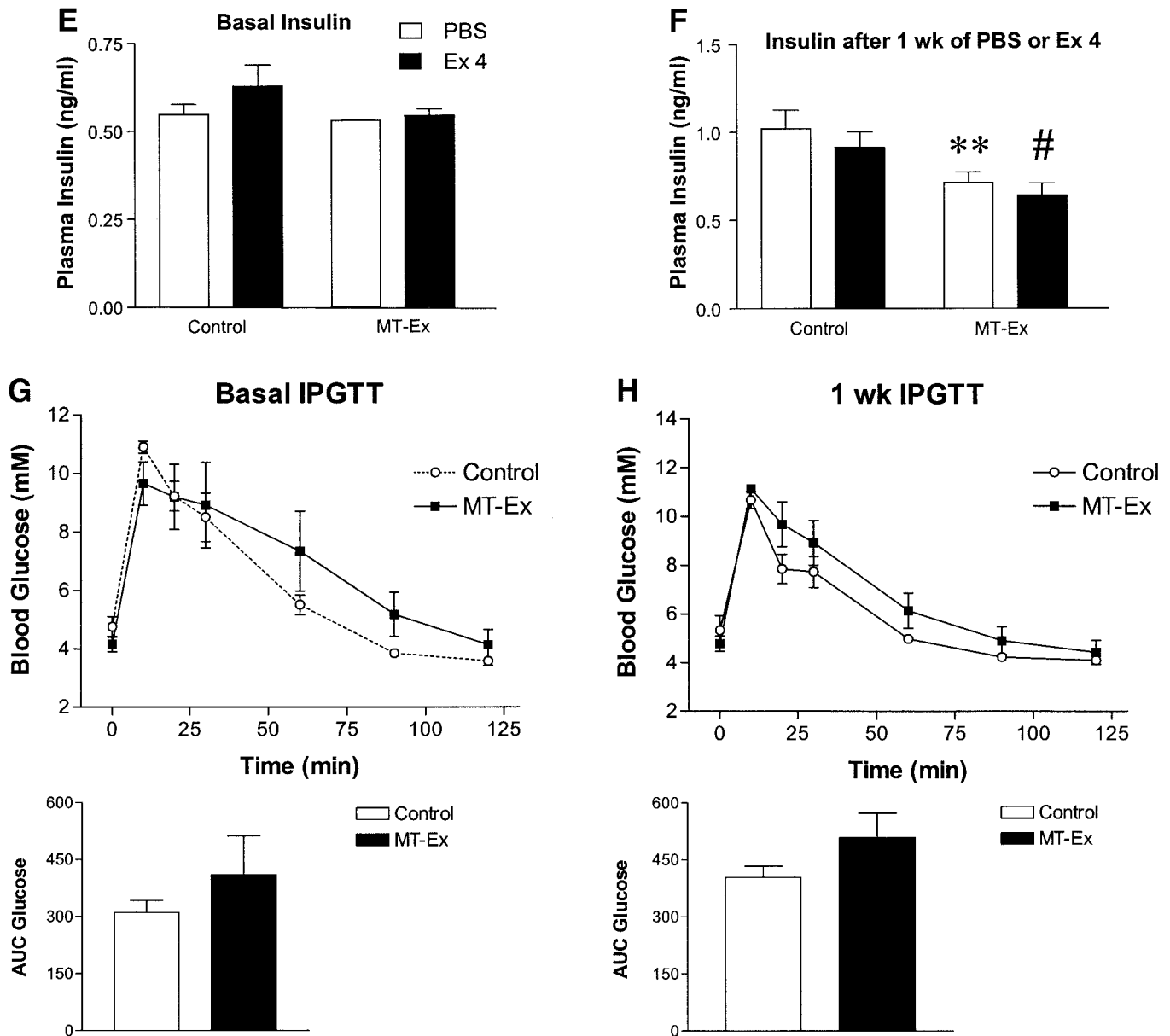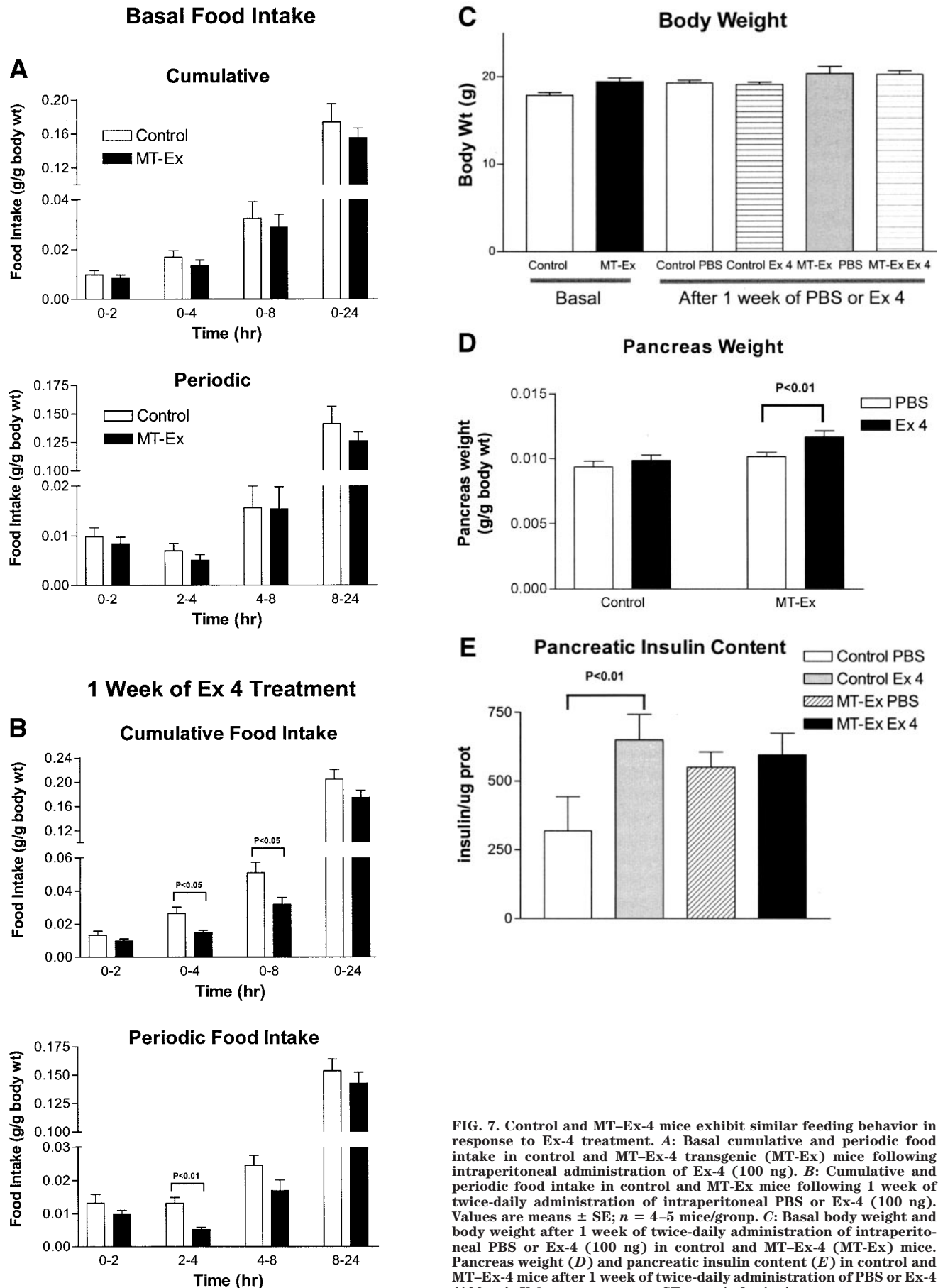
FIG. 6—Continued.

desensitization at their respective receptors in islet cell studies.

The rapid degradation of native GLP-1 by dipeptidyl peptidase IV has stimulated considerable interest in the development of degradation-resistant long-acting GLP-1 receptor agonists, such as Ex-4 (2). Surprisingly, there is little available data examining whether Ex-4 exhibits differences, relative to native GLP-1, in the magnitude of homologous GLP-1 receptor desensitization. Our findings using islet cells in vitro demonstrate that exposure to Ex-4 is associated with a greater degree of GLP-1 receptor desensitization compared with comparable incubations with GLP-1. Given the greater stability and prolonged activity of Ex-4 relative to native GLP-1 (25), it seems likely that islet cell GLP-1 receptors are exposed for a greater period of time to intact degradation-resistant Ex-4. No significant differences in receptor binding or postreceptor activation have been observed for Ex-4 relative to

GLP-1 (26) in vitro; however, Ex-4 is far more potent and exhibits an extended pharmacokinetic profile relative to native GLP-1 in vivo (25,27).

Several previous experiments have assessed the effects of continuous intracerebroventricular administration of GLP-1 on food intake. Chronic or intermittent intracerebroventricular GLP-1 administration inhibited food intake and reduced weight gain in Sprague Dawley and Zucker rats (28), whereas repeated intracerebroventricular GLP-1 inhibited and exendin (9-39) stimulated food intake in ad libitum fed rats (29). Similarly, chronic intermittent administration of GLP-1 receptor agonists to diabetic rodents is associated with inhibition of food intake, improvement in glycemia, and reduction in HbA_{1c}, demonstrating that repeated administration of structurally distinct GLP-1 receptor agonists is not associated with significant tachyphylaxis or a diminished therapeutic effect in vivo (3,30,31). In contrast, we did not observe differences in feeding behav-

Basal Food Intake

A

Cumulative

Periodic

1 Week of Ex 4 Treatment

B

Cumulative Food Intake

Periodic Food Intake

C

Body Weight

D

Pancreas Weight

E ### Pancreatic Insulin Content

FIG. 7. Control and MT–Ex-4 mice exhibit similar feeding behavior in response to Ex-4 treatment. *A*: Basal cumulative and periodic food intake in control and MT–Ex-4 transgenic (MT-Ex) mice following intraperitoneal administration of Ex-4 (100 ng). *B*: Cumulative and periodic food intake in control and MT-Ex mice following 1 week of twice-daily administration of intraperitoneal PBS or Ex-4 (100 ng). Values are means ± SE; *n* = 4–5 mice/group. *C*: Basal body weight and body weight after 1 week of twice-daily administration of intraperitoneal PBS or Ex-4 (100 ng) in control and MT–Ex-4 (MT-Ex) mice. Pancreas weight (*D*) and pancreatic insulin content (*E*) in control and MT–Ex-4 mice after 1 week of twice-daily administration of PBS or Ex-4 (100 ng). Values are means ± SE; *n* = 4–8 mice/group.

ior or body weight in control versus MT–Ex-4 transgenic mice either before or after a 1-week treatment period. Moreover, MT–Ex-4 transgenic mice appeared significantly more sensitive, relative to wild-type controls, to the anorexic effects of exogenous Ex-4 in vivo. Hence, central GLP-1 receptor systems important for transducing the satiety effects of GLP-1 receptor agonists do not appear to undergo significant functional downregulation in vivo.

Our studies examining the potential for Ex-4 to produce GLP-1 receptor desensitization were prompted in part by the phenotype of MT–Ex-4 transgenic mice (14). MT–Ex-4 mice correctly process preproexendin to biologically active Ex-4 (32) and exhibit no obvious disturbances in growth, feeding behavior, islet size, or basal glucose homeostasis, despite producing circulating levels of Ex-4 from birth that would be predicted to be associated with therapeutic GLP-1 receptor–dependent actions in vivo (14). The results of our current experiments demonstrate that MT–Ex-4 mice exhibit comparatively reduced (relative to wild-type control) acute glycemic responses to initial rechallenge with Ex-4 following an OGTT but not following IPGTT. These findings raise the possibility that MT–Ex-4 mice exhibit modest but detectable differences in the degree of GLP-1 receptor desensitization at the levels of gastric emptying versus insulin secretion in vivo. Nevertheless, we did not observe detectable differences in the glycemic response of wild-type versus MT–Ex-4 mice following 1 week of Ex-4 administration. Hence, these findings argue against the development of significant GLP-1 receptor desensitization coupled to control of glucose homeostasis in vivo.

Intriguingly, we detected increased pancreatic insulin content in wild-type but not in MT–Ex-4 transgenic mice following 1 week of Ex-4 administration. Somewhat surprisingly, however, we did not detect evidence for differences in the levels of GLP-1 receptor–dependent mRNA transcripts in wild-type versus MT–Ex-4 transgenic mice, and no changes in the levels of these transcripts were observed after 1 week of twice-daily Ex-4 administration. Hence, upregulation of "classic" GLP-1–regulated genes such as pdx-1 or insulin is not an invariable consequence following Ex-4 administration to normoglycemic mice.

The realization that sustained exposure to GLP-1 receptor agonists may produce therapeutically desirable effects on glucose control and energy homeostasis in subjects with type 2 diabetes (2) raises the question of whether continuous GLP-1 receptor activation might potentially be associated with clinically meaningful desensitization of the GLP-1 receptor in vivo. A 6-week study of native GLP-1 administered by continuous subcutaneous infusion produced significant reductions in fasting and postprandial glucose in association with reductions in HbA_{1c} and body weight (33). Similar glucose-lowering results were obtained in a study of eight subjects treated with continuous subcutaneous GLP-1 infusion for 12 weeks (8). Hence, sustained elevations in levels of circulating GLP-1 are not associated with a detectable loss of biological activity in vivo. Similarly, twice-daily administration of Ex-4 in subjects with type 2 diabetes produced a significant reduction in fructosamine and HbA_{1c} over a 12-week study period (13). Hence, although our studies clearly demonstrate that

the GLP-1 receptor exhibits the capacity for receptor desensitization following continuous exposure to GLP-1 receptor agonists in vitro, the available evidence clearly indicates that continuous activation of the GLP-1 receptor in diabetic subjects does not lead to a detectable loss of GLP-1 receptor–dependent biological activity in vivo. Nevertheless, as GLP-1 receptro agonists do not achieve a normalization of fasting or postprandial glucose in all treated diabetic subjects, increased understanding of the molecular determinants linking GLP-1 receptor activation to sustained lowering of blood glucose may be useful for optimizing therapeutic outcomes with these agents in future studies.

ACKNOWLEDGMENTS

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