Original Article

Chemical Ablation of Gastric Inhibitory Polypeptide Receptor Action by Daily (Pro^3)GIP Administration Improves Glucose Tolerance and Ameliorates Insulin Resistance and Abnormalities of Islet Structure in Obesity-Related Diabetes

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Glucose-dependent insulinotropic polypeptide (gastric inhibitory polypeptide [GIP]) is an important incretin hormone secreted by endocrine K-cells in response to nutrient ingestion. In this study, we investigated the effects of chemical ablation of GIP receptor (GIP-R) action on aspects of obesity-related diabetes using a stable and specific GIP-R antagonist, (Pro^3)GIP. Young adult *ob/ob* mice received once-daily intraperitoneal injections of saline vehicle or (Pro^3)GIP over an 11-day period. Nonfasting plasma glucose levels and the overall glycemic excursion (area under the curve) to a glucose load were significantly reduced (1.6-fold; $P < 0.05$) in (Pro^3)GIP-treated mice compared with controls. GIP-R ablation also significantly lowered overall plasma glucose (1.4-fold; $P < 0.05$) and insulin (1.5-fold; $P < 0.05$) responses to feeding. These changes were associated with significantly enhanced (1.6-fold; $P < 0.05$) insulin sensitivity in the (Pro^3)GIP-treated group. Daily injection of (Pro^3)GIP reduced pancreatic insulin content (1.3-fold; $P < 0.05$) and partially corrected the obesity-related islet hypertrophy and β-cell hyperplasia of *ob/ob* mice. These comprehensive beneficial effects of (Pro^3)GIP were reversed 9 days after cessation of treatment and were independent of food intake and body weight, which were unchanged. These studies highlight a role for GIP in obesity-related glucose intolerance and emphasize the potential of specific GIP-R antagonists as a new class of drugs for the alleviation of insulin resistance and treatment of type 2 diabetes. *Diabetes* 54:2436–2446, 2005

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AUC, area under the curve; DPP, dipeptidyl peptidase; GIP, glucose-dependent insulinotropic polypeptide; GIP-R, GIP receptor; GLP-1, glucagon-like peptide 1.

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Glucose-dependent insulinotropic polypeptide (gastric inhibitory polypeptide [GIP]) is a 42–amino acid peptide hormone secreted by enteroendocrine K-cells after nutrient absorption (1). In the pancreas, GIP stimulates glucose-dependent insulin secretion through interaction with specific heterotrimeric G-protein–coupled GIP receptors (GIP-Rs) on pancreatic β-cells (2). Evidence suggests that GIP also stimulates proinsulin gene transcription and translation (3) and acts as a β-cell growth factor (4,5) and antiapoptotic agent (6,7). Receptors for GIP have been demonstrated at a number of extrapancreatic sites (8,9), suggesting a range of additional effects on nutrient metabolism. Key among these is the ability of GIP to inhibit gastric acid secretion (10), attenuate glucagon-stimulated hepatic glucose production (11), stimulate glucose uptake in muscle (12), and increase both fatty acid synthesis and lipoprotein lipase activity in adipocytes (13,14). This action profile is reminiscent, but not identical, to that displayed by glucagon-like peptide 1 (GLP-1) (15). Together, GIP and GLP-1 constitute two major physiological incretin hormones of the enteroinsular axis (16).

Much recent attention has been devoted to enhancement of incretin action using dipeptidyl peptidase (DPP) IV inhibitors or stable analogs of GLP-1 and GIP for the treatment of type 2 diabetes (17,18). Such an approach is reliant on the possibility that incretin action is defective in diabetes and that the underlying defects responsible for metabolic disarray might be overridden by exogenous GLP-1 or GIP administration. There is now mounting evidence for a beneficial and possibly therapeutic role of both GLP-1 and GIP analogs in diabetes (1,8,16–18). Nevertheless, understanding of the possible involvement of incretin hormones in the pathophysiology of diabetes is lacking, partly because of cross-reaction of classical GLP-1 and GIP radioimmunoassays with the predominant DPP

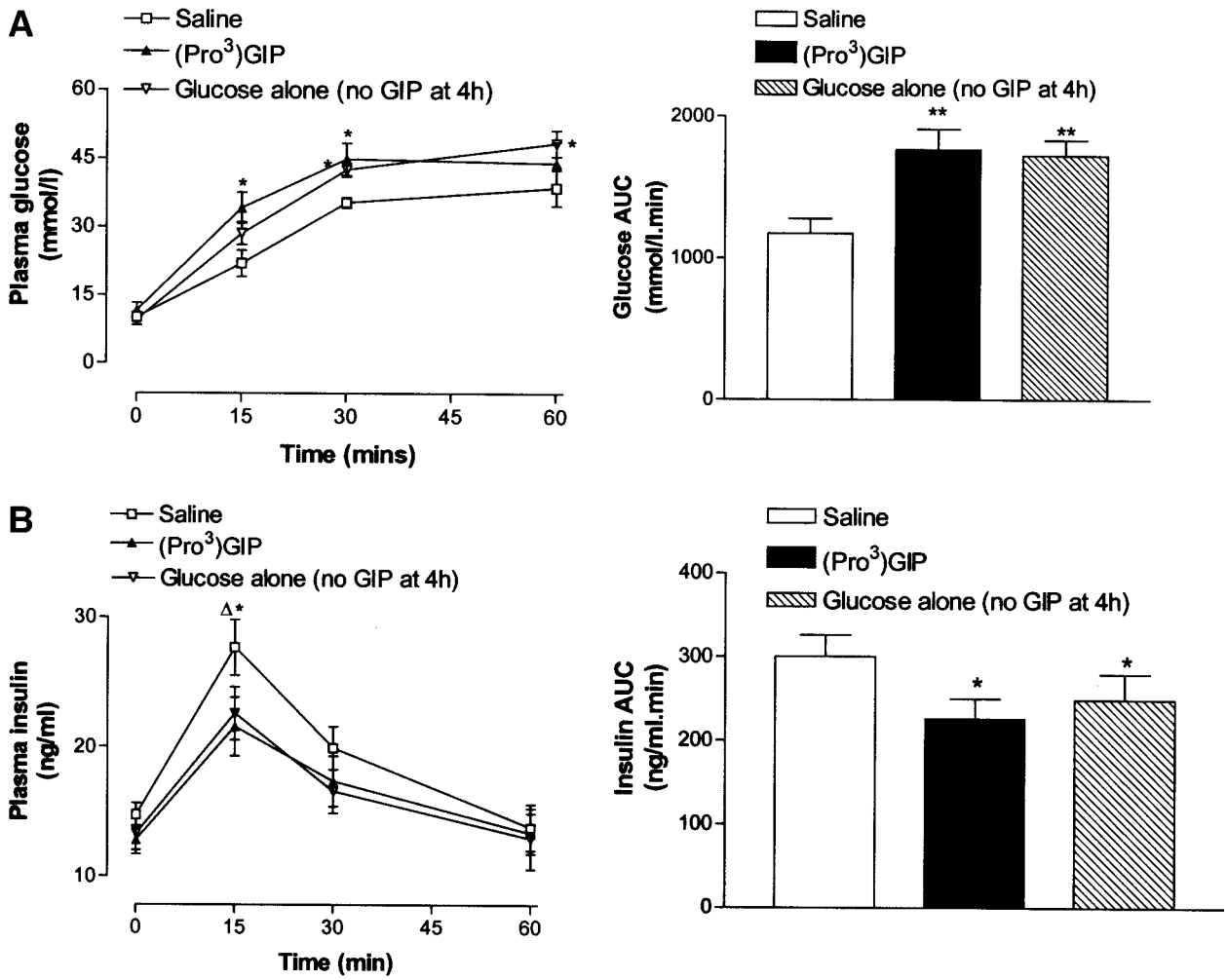

FIG. 1. Effects of (Pro^3)GIP on plasma glucose and insulin response to native GIP 4 h after administration. Tests were conducted 4 h after administration of (Pro^3)GIP (25 nmol/kg body wt) or saline (0.9% NaCl) in 18-h–fasted *ob/ob* mice. Plasma glucose and insulin concentrations were measured before and after intraperitoneal administration of glucose (18 mmol/kg body wt) alone or in combination with native GIP (25 nmol/kg body wt). The incremental areas under the glucose or insulin curves (AUC) between 0 and 60 min are shown in the *right panels*. Values represent means ± SE for eight mice. *$P < 0.05$ and **$P < 0.01$ compared with saline group.

IV–generated truncated peptide forms, GLP-1 (9–36) and GIP (3–42), which circulate at particularly high concentrations (1). Current opinion from clinical studies seems to suggest existence of a defect in the secretion of GLP-1 and a defect in the action of GIP in type 2 diabetes (16). However, the basis for such a conclusion is not impressive given the many previous contradictory human studies (19) and the likelihood that the reported insensitivity of pancreatic β-cells to GIP (20) may reflect a generalized secretory dysfunction rather than a specific cellular defect (21). The insulin secretory response to all secretagogues, including GLP-1, is compromised in type 2 diabetes (22,23). Thus the proposed use of GLP-1 and GIP for diabetes therapy is reliant on peptide engineering to provide analogs of incretin hormones with improved potency due to DPP IV resistance, decreased renal clearance, and/or enhanced GIP receptor and postreceptor activity (8).

Although no single animal model can match the complex etiology of type 2 diabetes in humans, studies of the *ob/ob* syndrome in mice have highlighted notable abnormalities of GIP in relation to the interplay between hyperphagia, hyperinsulinemia, and the metabolic demise associated with progressive obesity-related diabetes (24–26). These animals constitute a model of type 2 diabetes associated with gross obesity and severe insulin resistance, driven by leptin deficiency (27). Furthermore, recent research suggests that an interaction between leptin and the enteroinsular axis exists (28) and that overstimulation of the GIP-R on adipocytes appears to be an important contributor to fat deposition in *ob/ob* mice (29).

In this study, we have used daily injections of the stable and specific GIP-R antagonist, (Pro^3)GIP, to chemically ablate the GIP-R and evaluate the role of endogenous circulating GIP in obesity-related diabetes as manifested in *ob/ob* mice. The results reveal a cardinal role for GIP in insulin resistance and associated metabolic disturbances, and provide the first experimental evidence that GIP-R antagonists might provide a novel and effective means of treating obesity-driven forms of type 2 diabetes.

RESEARCH DESIGN AND METHODS

Obese diabetic (*ob/ob*) mice derived from the colony maintained at Aston University, Birmingham, U.K. (30), were used at 12–16 weeks of age. Animals were age matched, divided into groups, and housed individually in an air-conditioned room at 22 ± 2°C with a 12-h–light:12-h–dark cycle. Drinking water and a standard rodent maintenance diet (Trouw Nutrition, Cheshire,

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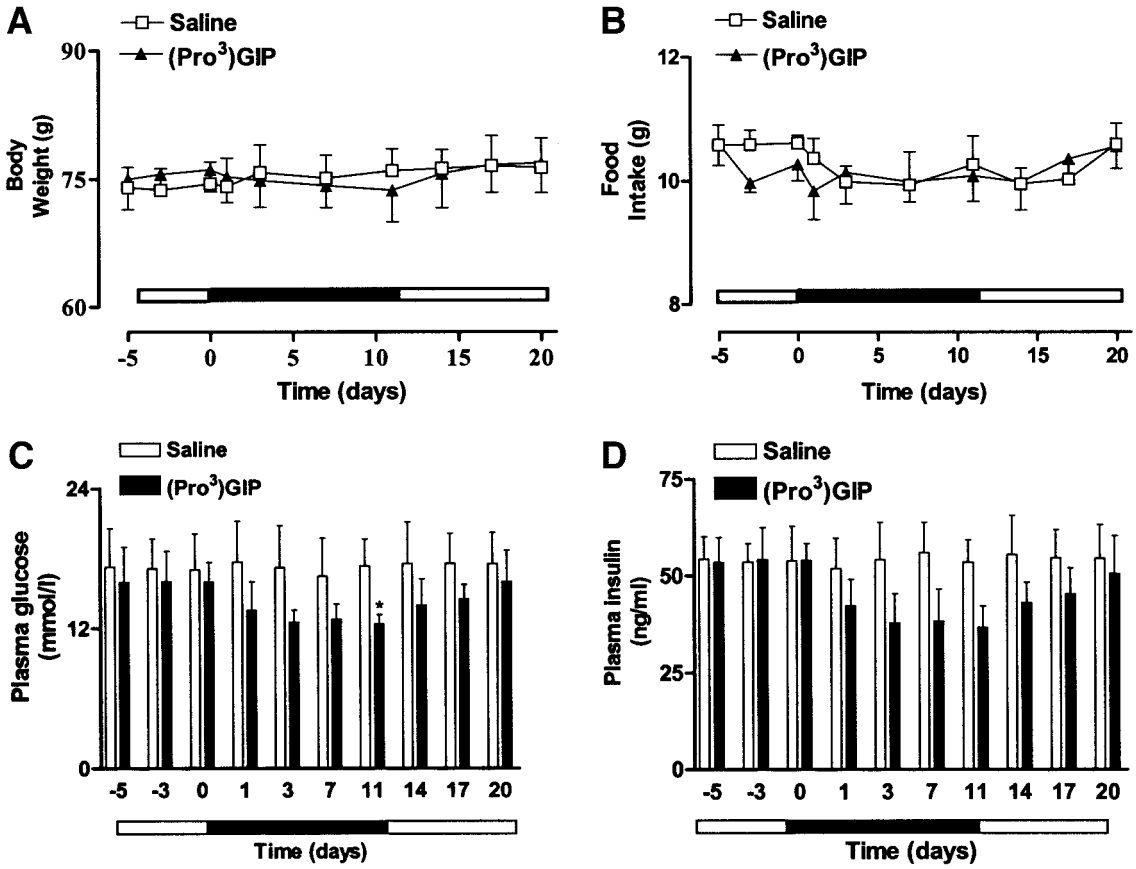

FIG. 2. Effects of daily (Pro^3)GIP administration on food intake (*A*), body weight (*B*), plasma glucose (*C*), and insulin concentrations (*D*) of *ob/ob* mice. Parameters were measured for 5 days before, 11 days during (indicated by horizontal black bar), and 9 days after treatment with saline or (Pro^3)GIP (25 nmol/kg body wt/day). Values are means ± SE for eight mice. *$P < 0.05$ compared with saline group.

U.K.) were freely available. All animal experiments were carried out in accordance with the U.K. Animals (Scientific Procedures) Act 1986. No adverse effects were observed after administration of (Pro^3)GIP.

Synthesis, purification, and characterization of (Pro^3)GIP. (Pro^3)GIP was sequentially synthesized on an Applied Biosystems automated peptide synthesizer (model 432 A) as reported previously (31). (Pro^3)GIP was purified by reverse-phase high-performance liquid chromatography on a Waters Millennium 2010 chromatography system (software version 2.1.5) and subsequently characterized using electrospray ionization mass spectrometry as described previously (31).

Experimental protocols for *ob/ob* mouse studies. Initially, extended biological activity of (Pro^3)GIP was examined in 18-h–fasted *ob/ob* mice 4 h after administration. Thereafter, over an 11-day period, mice received once-daily intraperitoneal injections (1700) of either saline vehicle (0.9% [wt/vol] NaCl) or (Pro^3)GIP (25 nmol/kg body wt). During a subsequent 9-day period, observations were continued after discontinuation of (Pro^3)GIP administration. Food intake and body weight were recorded daily, whereas plasma glucose and insulin concentrations were monitored (1000) at intervals of 2–6 days. Whole blood for the measurement of HbA_{1c} (A1C) was taken on days 11 and 20. Intraperitoneal glucose tolerance (18 mmol/kg body wt), metabolic response to native GIP (25 nmol/kg body wt), and insulin sensitivity (50 units/kg body wt) tests were performed on days 11 and 20. Mice fasted for 18 h were used to examine the metabolic response to 15 min of feeding. All acute tests commenced at 1000. In a separate series, pancreatic tissues were excised at the end of the 11-day treatment period or 9 days after discontinuation of (Pro^3)GIP and processed for immunohistochemistry or measurement of insulin after extraction with 5 ml/g ice-cold acid ethanol (750 ml ethanol, 235 ml H_2O, and 15 ml of concentrated HCl). Blood samples taken from the cut tip of the tail vein of conscious mice at the times indicated in the figures were immediately centrifuged using a Beckman microcentrifuge (Beckman Instruments, Galway, Ireland) for 30 s at 13,000*g*. The resulting plasma was then aliquoted into fresh Eppendorf tubes and stored at −20°C before glucose and insulin determinations.

Biochemical analysis. Plasma glucose was assayed by an automated glucose oxidase procedure (32) using a Beckman Glucose Analyzer II (Beckman Instruments). Plasma and pancreatic insulin were assayed by a modified dextran-coated charcoal radioimmunoassay (33). A1C was determined using cation-exchange columns (Sigma, Poole, Dorset, U.K.) with measurement of absorbance (415 nm) in wash and eluting buffer using a VersaMax Microplate Spectrophotometer (Molecular Devices, Wokingham, Berkshire, U.K.).

Immunocytochemistry. Tissue fixed in 4% paraformaldehyde–PBS and embedded in paraffin was sectioned at 8 μm. After dewaxing, sections were incubated with blocking serum (Vector Laboratories, Burlingame, CA) before exposure to insulin antibody. Tissue samples were then incubated consecutively with secondary biotinylated universal, pan-specific antibody (Vector Laboratories) and streptavidin-peroxidase preformed complex (Vector Laboratories). After washing, the stained pancreatic tissue was counterstained with hematoxylin (BDH Chemicals, Dorset, U.K.) and then plunged into acid methanol (500 ml methanol, 500 ml H_2O, and 2.5 ml concentrated HCl) before dehydration and mounting in Depex (BDH Chemicals). The stained slides were viewed under a microscope (Nikon Eclipse E2000; Diagnostic Instruments, Sterling Heights, MI) attached to a JVC camera (model KY-F55B; JVC, London, U.K.) and analyzed using Kromoscan imaging software (Kinetic Imaging Limited, Faversham, Kent, U.K.). The average number and diameter of every islet in each section was estimated in a blinded manner using an eyepiece graticule calibrated with a stage micrometer (Graticules Limited, Tonbridge, Kent, U.K.). The longest and shortest diameters of each islet were determined with the graticule. Half of the sum of these two values was then considered to be the average islet diameter. Approximately 60–70 random sections were examined from the pancreas of each mouse.

Statistics. Results are expressed as means ± SE. Data were compared using ANOVA, followed by a Student-Newman-Keuls post hoc test. Area under the curve (AUC) analyses were calculated using the trapezoidal rule with baseline subtraction (34). $P < 0.05$ was considered to be statistically significant.

RESULTS

Effects of (Pro^3)GIP on plasma glucose and insulin concentrations 4 h after administration. As shown in

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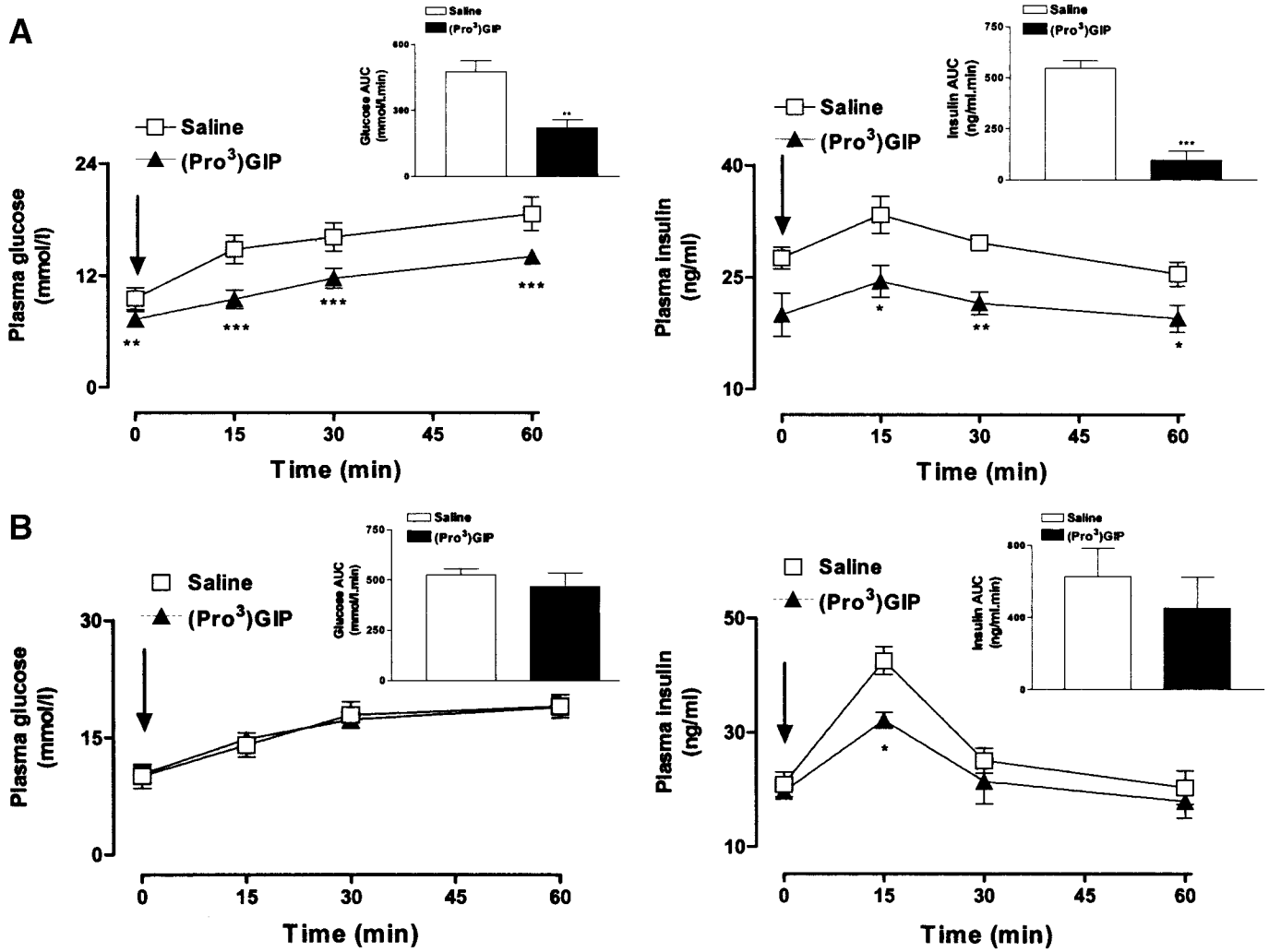

FIG. 3. Effects of daily (Pro^3)GIP administration on glucose tolerance and plasma insulin response to glucose in *ob/ob* mice. Tests were conducted after daily treatment with (Pro^3)GIP (25 nmol/kg body wt/day) for 11 days (*A*) or 9 days after cessation of treatment (*B*). Glucose (18 mmol/kg body wt) was administered at the time indicated by the arrow. Plasma glucose and insulin AUC values for 0–60 min postinjection, with identical baseline subtractions in each case to demonstrate the complete effect of (Pro^3)GIP treatment, are shown in *insets*. Values are means ± SE for eight mice. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared with saline group.

Fig. 1, administration of (Pro^3)GIP 4 h previously impaired the plasma glucose and insulin responses to native GIP, given together with glucose. AUC glucose and insulin values were increased by 151% ($P < 0.05$) and decreased by 25% ($P < 0.05$), respectively, compared with saline-treated controls. The resulting profile was similar to that induced by glucose given alone in the absence of GIP. This supports a protracted biological half-life and forms the basis of the once-daily injection. Unfortunately, it is not possible to measure (Pro^3)GIP in plasma of *ob/ob* mice using commercially available GIP antiserum. However, it is notable that exenatide, a DPP IV–resistant synthetic agonist of the sister incretin hormone GLP-1, persists for more than 15 h after a single injection in patients with type 2 diabetes (35).

Effects of (Pro^3)GIP on food intake, body weight, and nonfasting plasma glucose and insulin concentrations. Administration of (Pro^3)GIP had no effect on food intake and body weight (Fig. 2*A* and *B*). On day 11, plasma glucose had declined to significantly reduced ($P < 0.05$) concentrations in *ob/ob* mice receiving (Pro^3)GIP (Fig. 2*C*). Cessation of treatment returned plasma glucose concentrations toward control levels. Consistent with this pattern, A1C was significantly lower ($P < 0.05$) after 11 days of treatment with (Pro^3)GIP than either before or 9 days after cessation of daily injection (8.0 ± 0.3, 6.9 ± 0.2, and 7.7 ± 0.4%, respectively). No significant changes in plasma insulin levels were noted during or after the treatment period (Fig. 2*D*).

Effects of (Pro^3)GIP on glucose tolerance. Daily administration of (Pro^3)GIP for 11 days resulted in significantly reduced ($P < 0.001$) plasma glucose concentrations at 15, 30, and 60 min after intraperitoneal glucose (Fig. 3*A*). This was corroborated by a significantly decreased 0- to 60-min AUC value (Fig. 3*A*), which was reduced 2.1-fold ($P < 0.01$) compared with controls. Plasma insulin concentrations were also significantly ($P < 0.05$) reduced 15, 30, and 60 min after intraperitoneal glucose injection in the (Pro^3)GIP-treated group (Fig. 3*A*). AUC 0- to 60-min values were also significantly decreased ($P < 0.001$). Interestingly, an almost identical pattern was observed when 11-day–treated *ob/ob* mice were administered glucose together with native GIP (25 nmol/kg body wt) (Fig. 4). This supports the view that GIP action was effectively antagonized in the (Pro^3)GIP-treated group. Discontinuation of

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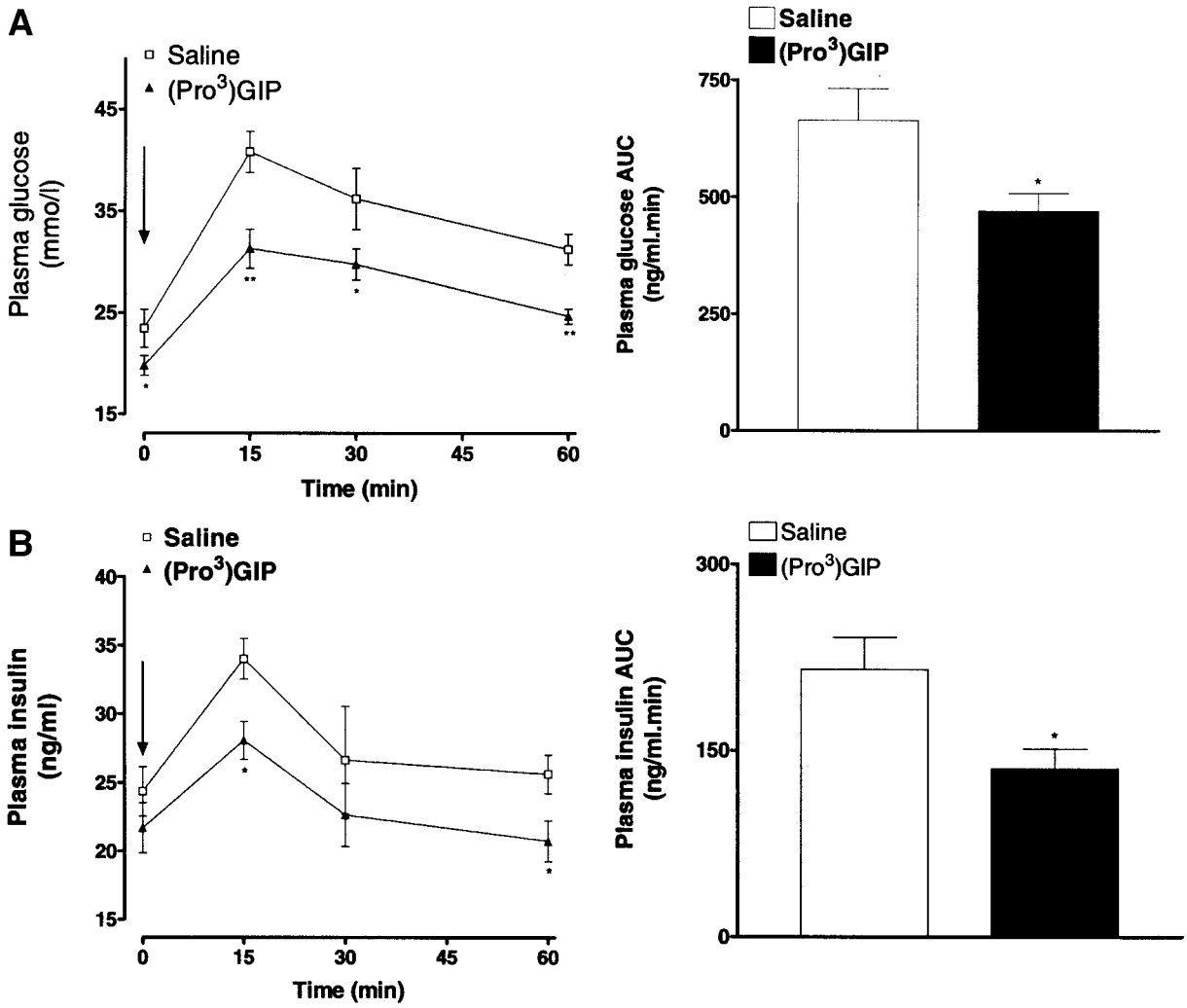

FIG. 4. Effects of daily (Pro^3)GIP administration on plasma glucose and insulin responses to native GIP administered together with glucose. Tests were conducted after treatment with (Pro^3)GIP (25 nmol/kg body wt/day) or saline for 11 days. GIP (25 nmol/kg body wt) and glucose (18 mmol/kg body wt) were administered at the time indicated by the arrow. Plasma glucose and insulin AUC values for 0–60 min are shown in the *right panels*. Values represent means ± SE for eight mice. *$P < 0.05$ and **$P < 0.01$ compared with saline group.

(Pro^3)GIP treatment for 9 days (20th day of study) resulted in almost identical plasma glucose and insulin responses to intraperitoneal glucose (Fig. 3), with lower glucose-mediated plasma insulin concentrations noted at one time point (15 min; $P < 0.05$).

Effects of (Pro^3)GIP on metabolic response to feeding and insulin sensitivity. Plasma glucose and insulin responses to 15-min feeding were significantly lowered ($P < 0.05$) at 30 and 60 min in *ob/ob* mice treated with (Pro^3)GIP for 11 days (Fig. 5). Similarly, AUC glucose and insulin were significantly ($P < 0.05$) decreased in (Pro^3)GIP-treated *ob/ob* mice, despite similar food intakes of 0.3–0.5 g · mouse^{-1} · 15 min^{-1}. As shown in Fig. 6*A*, the hypoglycemic action of insulin was significantly ($P < 0.05$) augmented in terms of AUC measures and postinjection values in *ob/ob* mice treated with (Pro^3)GIP for 11 days. The responses 9 days after discontinuation of (Pro^3)GIP treatment were similar to those in saline-treated controls (Fig. 6*B*).

Effects of (Pro^3)GIP on pancreatic insulin and islet morphology. (Pro^3)GIP treatment had no effect on pancreatic weight (Fig. 7*A*). However, pancreatic insulin content was significantly ($P < 0.05$) decreased in *ob/ob* mice receiving (Pro^3)GIP for 11 days compared with controls (Fig. 7*B*). No significant differences were observed in islet number per pancreatic section (Fig. 7*C*), but average islet diameter was markedly and significantly reduced ($P < 0.001$) in (Pro^3)GIP-treated *ob/ob* mice (Fig. 7*D*). These effects were effectively reversed by discontinuation of (Pro^3)GIP on day 20; however, average islet diameter was still significantly reduced ($P < 0.05$). As shown in Fig. 8*A*, more detailed analysis revealed that the reduction is islet diameter on day 11 was due to a significant decrease ($P < 0.001$) in the percentage of larger diameter (>0.15 mm) islets with increases in the proportion of islets with small (<0.10 mm) and medium (0.1- 0.15 mm) diameters. Figure 8*D* presents similar analysis after cessation of treatment, with a significant ($P < 0.05$) increase in the percentage of small islets still apparent. Representative images (magnification ×40) of pancreata immunohistologically stained for insulin from 11-day (Pro^3)GIP-treated *ob/ob* mice (Fig. 8*B*) and saline-treated controls (Fig. 8*C*) illustrate the dramatic changes in pancreatic islet morphology induced by (Pro^3)GIP treatment. Pancreata immunohistologically stained for insulin on day 20 are also shown (Fig. 8*E* and *F*).

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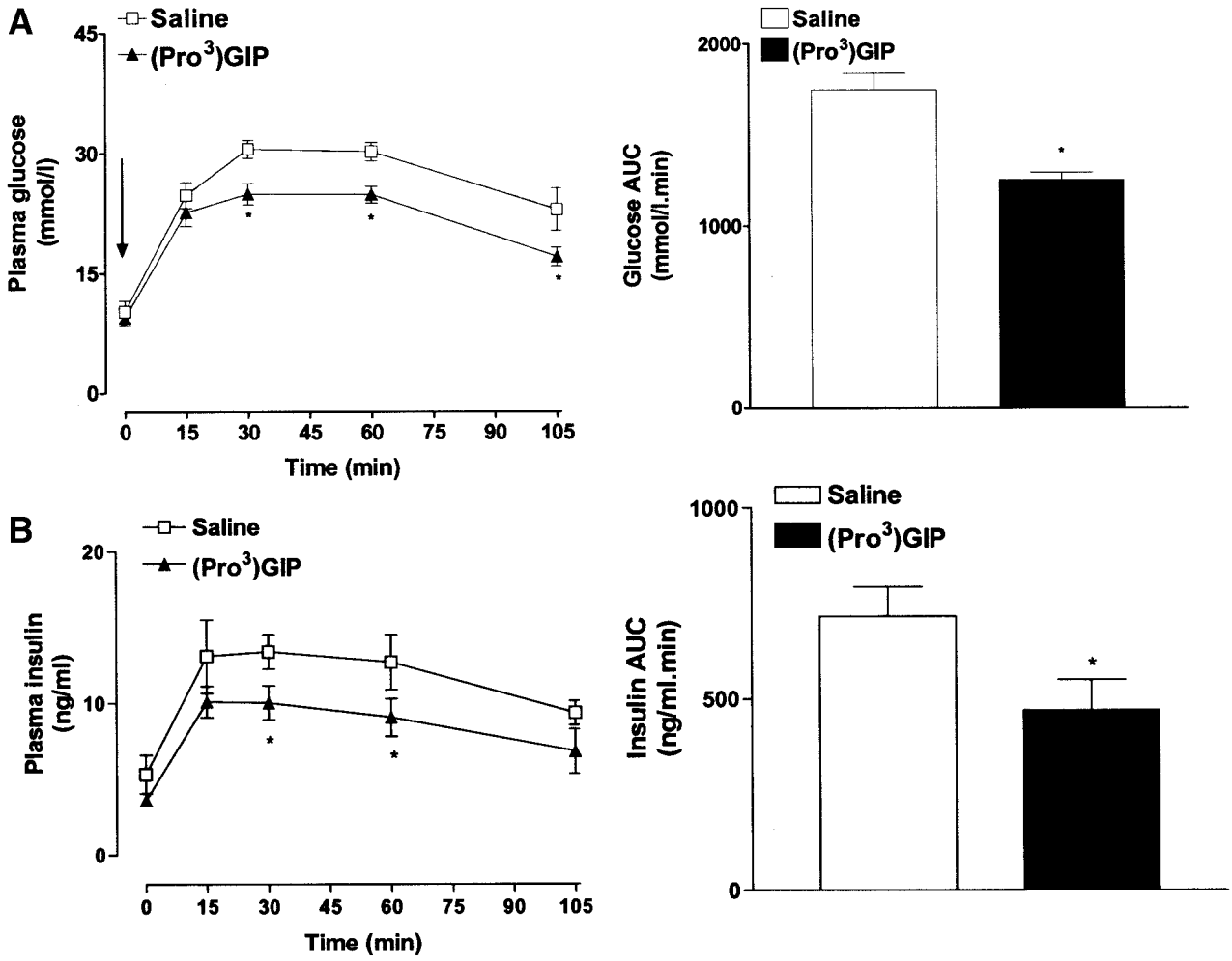

FIG. 5. Effects of daily (Pro^3)GIP administration on glucose and insulin responses to feeding in 18-h–fasted *ob/ob* mice. Tests were conducted after daily treatment with (Pro^3)GIP (25 nmol/kg body wt/day) or saline for 11 days. The arrow indicates the time of feeding (15 min). AUC values for 0–105 min postfeeding are also shown. Values are means ± SE for eight mice. *P < 0.05 compared with saline group.

DISCUSSION

Knockout of the GIP-R in normal mice has been shown to result in significant impairment of glucose tolerance and meal-induced insulin secretion without appreciable effects on food intake, body weight, basal glucose, or insulin concentrations (36). More recent studies with genetic GIP-R knockout mice have corroborated these findings and have additionally shown that GIP has a significant involvement in the enteroinsular axis (37,38). However, double knockout of receptors for GLP-1 and GIP results in a surprisingly modest deterioration of glucose homeostasis (39,40), indicating possible upregulation of compensatory mechanisms during life-long deletion of GLP-1 and GIP receptors.

We have recently developed (Pro^3)GIP as a specific and potent antagonist of the GIP-R that is highly stable and resistant to DPP IV–mediated degradation (31). Using (Pro^3)GIP acutely, we have highlighted the involvement of GIP in the plasma insulin response to feeding and the enteroinsular axis of *ob/ob* mice (41). Comparison with the effects of the GLP-1-R antagonist, exendin (9–39), indicates that GIP contributes substantially to the insulin-releasing actions of the enteroinsular axis and represents the major physiological incretin (41). Consistent with this view, we have recently shown that once-daily administration of (Pro^3)GIP to normal mice for 11 days results in the reversible impairment of glucose tolerance associated with decreased insulin sensitivity (42). Basal and postprandial insulin secretion together with pancreatic insulin content and islet morphology were unchanged. Thus, longer-term chemical ablation of GIP-R function with daily (Pro^3)GIP largely mimics the phenotype induced by genetic GIP-R knockout in mice with the exception of revealing a potentially important additional effect of endogenous GIP on insulin action, which appears to be independent of enhanced insulin secretion.

Given the important role ascribed to GIP in the enteroinsular axis and the fact that *ob/ob* mice are noted for intestinal K-cell hyperplasia and markedly elevated concentrations of intestinal and circulating GIP (24–26), it may be logical to anticipate that chemical ablation of GIP-Rs with (Pro^3)GIP would result in a significant worsening of glucose homeostasis and increasing hyperglycemia in *ob/ob* mice. Thus, considering the insulin-releasing actions of elevated GIP in young *ob/ob* mice and its likely link to the hyperinsulinemia of the mutant (24–26), an anticipated decline in circulating insulin as a consequence of GIP-R ablation would be expected to counteract the

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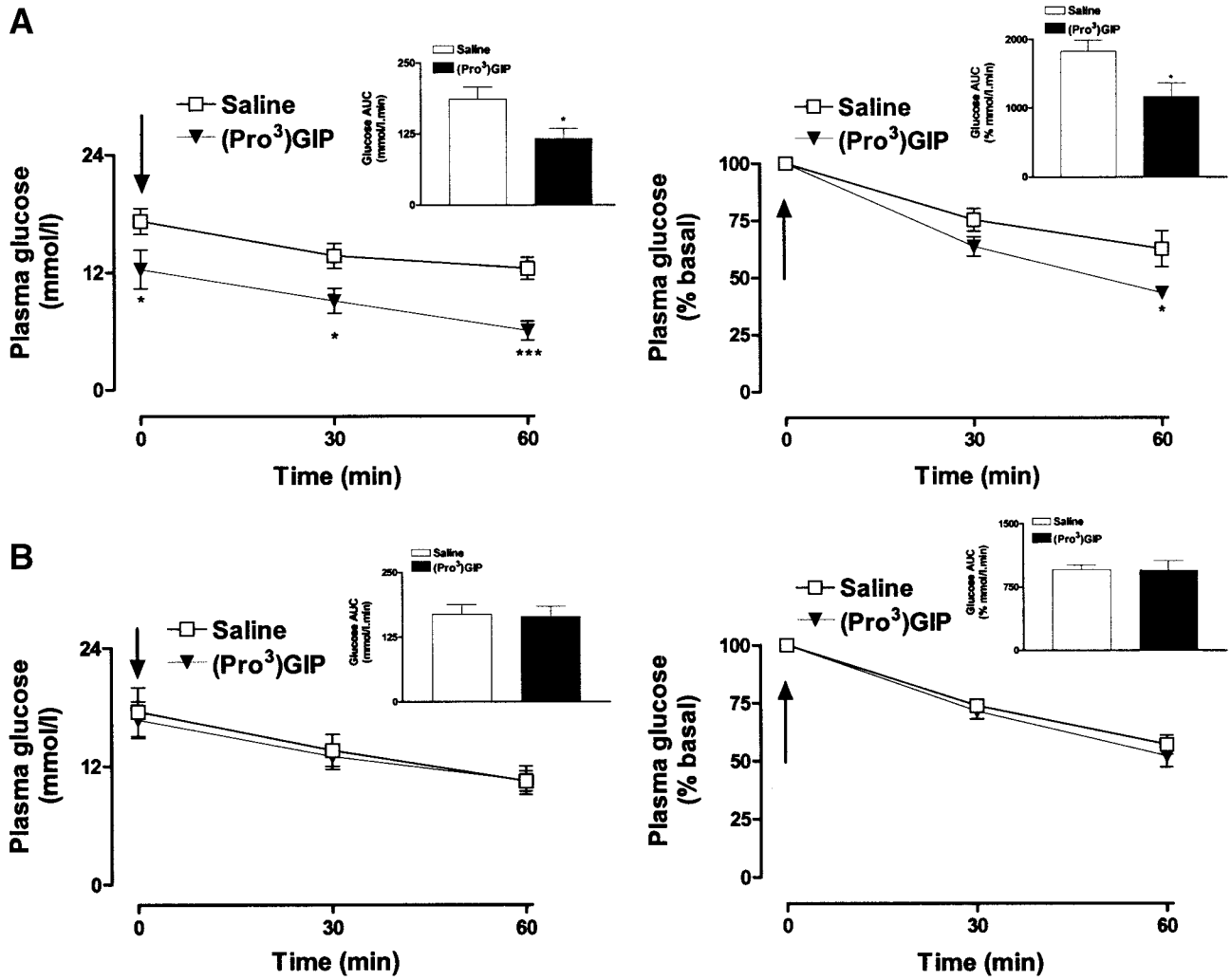

FIG. 6. Effects of daily (Pro^3)GIP administration on insulin sensitivity in *ob/ob* mice. Tests were conducted after daily treatment with (Pro^3)GIP (25 nmol/kg body wt/day) or saline for 11 days (*A*) or 9 days (*B*) after cessation of treatment. Data are presented as millimoles per liter (*left panels*) and percent basal (*right panels*). Insulin (50 units/kg body wt) was administered by intraperitoneal injection at the time indicated by the arrow. AUC values for 0–60 min postinjection are also shown. Values are means ± SE for eight mice.*$P < 0.05$ compared with saline group.

compensatory β-cell hyperplasia thought to lay behind the lack of progression of Aston *ob/ob* mice to the more severe diabetes phenotype expressed by the *ob* mutation on the C57BL/KsJ background (27).

Far from reproducing this predicted scenario and the metabolic deterioration observed after genetic or chemical knockout of the GIP-R in normal mice (36,42), *ob/ob* mice treated with daily (Pro^3)GIP for 11 days exhibited a marked improvement in diabetic status. This included decreased fasting and basal hyperglycemia, lowered A1C, improved glucose tolerance, and a significantly diminished glycemic excursion after feeding. Notably, basal and glucose-stimulated plasma insulin concentrations were decreased, suggesting that insulin sensitivity must have improved significantly after (Pro^3)GIP to restrain the hyperglycemia. Insulin sensitivity tests conducted after 11 days of (Pro^3)GIP administration revealed a 57% increase in the glucose-lowering action of exogenous insulin. Bearing in mind that the severity of the *ob/ob* syndrome represents a tough test for current antidiabetic drugs, including insulin, sulfonylureas, metformin, and thiazolidenediones (23,43,44), induction of such rapid and reversible changes by GIP-R blockade using (Pro^3)GIP is unprecedented.

It is important to note that the above effects were observed independently of any change in food intake or body weight in (Pro^3)GIP-treated *ob/ob* mice. This is in accordance with the view that endogenous GIP lacks effects on feeding activity (1). However, the observation on body weight contrasts with findings in *ob/ob* mice crossbred to genetically knockout GIP-R function (29). Thus in these transgenic mice, life-long depletion of GIP-R function was associated with decreased body weight gain and significant amelioration of both adiposity and insulin resistance compared with control (Lep^{ob}/Lep^{ob}) mice (29). In this previous study, the improvement of insulin sensitivity may have been a simple consequence of reduced adipose tissue mass, as this would significantly enhance peripheral glucose disposal (27). However, the present results observed in rapid time and without effects on feeding or body weight clearly indicate the involvement of an alternative mechanism.

The most plausible explanation for the present data stems from appreciation of the key milestones in the

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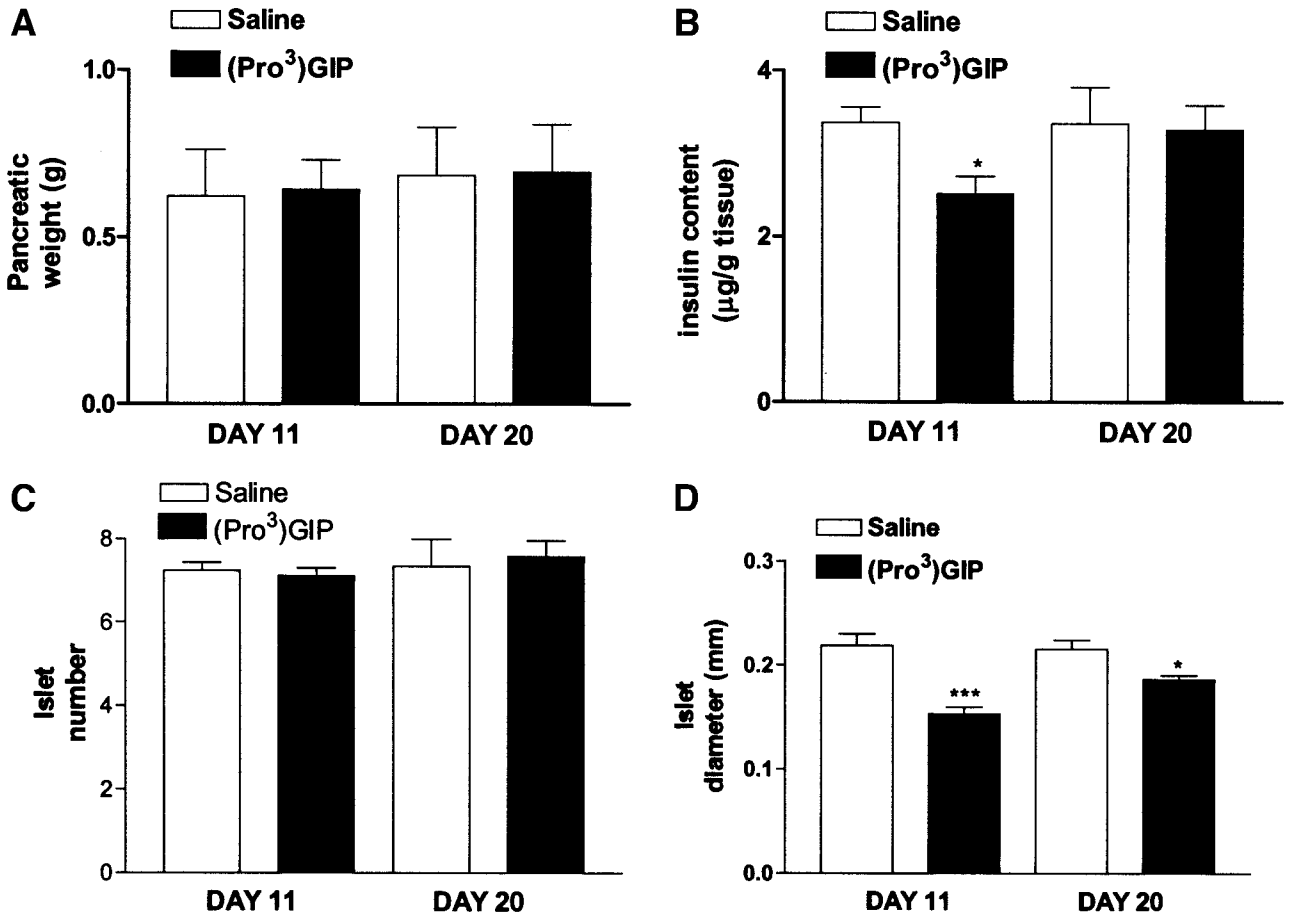

FIG. 7. Effects of daily (Pro^3)GIP administration on pancreatic weight (*A*), insulin content (*B*), islet number (*C*), and islet diameter (*D*) in *ob/ob* mice. Parameters were measured after daily treatment with (Pro^3)GIP (25 nmol/kg body wt/day) or saline for 11 days and 9 days after cessation of treatment (day 20). Values are means ± SE for eight mice. *$P < 0.05$ and ***$P < 0.001$ compared with saline group.

age-dependent progression of the *ob/ob* syndrome on the Aston background, as depicted in Fig. 9. Because of double-recessive *ob* mutation and resulting leptin deficiency, young *ob/ob* mice develop a profound early hyperphagia (30). Substantial enteroendocrine stimulation results in K-cell hyperplasia and markedly elevated concentrations of intestinal and circulating GIP (24–26). This in turn promotes islet hypertrophy and β-cell hyperplasia (30) together with marked hyperinsulinemia and mounting insulin resistance (45). This process manifests itself in terms of rising basal hyperglycemia and glucose intolerance. A vicious spiral is thus established wherein β-cell compensation results in marked hyperinsulinemia that attempts to moderate increasing insulin resistance (30,45). Viewed in this context, it is clear that chemical ablation of GIP-R function with daily (Pro^3)GIP will decrease β-cell stimulation and hyperinsulinemia. However, instead of causing further impairment of glucose homeostasis, a preferentially marked improvement of insulin sensitivity results in a substantial improvement of the metabolic syndrome. Further support for this scenario is the partial amelioration of islet hypertrophy and β-cell hyperplasia in (Pro^3)GIP-treated *ob/ob* mice (Fig. 8). Notably, average islet diameter was diminished with the largest islets (>15 mm) being replaced by a greater proportion with small or medium diameters (0.1–15 mm). These effects were largely reversed by 9-day cessation of treatment, supporting the idea of active islet and β-cell growth in adult *ob/ob* mice (30). Recent observations indicate that GIP acts as a mitotic stimulus and antiapoptotic agent to the β-cell (4–7). Thus, we suppose that negative effects of (Pro^3)GIP on islet size reflect a combination of decreased proliferation and increased apoptosis of β-cells. However, further studies are required to substantiate this view.

Although blockade of GIP-R action on β-cells may explain a large part of the observed changes induced by (Pro^3)GIP on islet architecture, plasma insulin, insulin sensitivity, and glucose homeostasis, it is unlikely to represent the entire story. For example, blockade of GIP upregulation of hexose transport in the intestine may be one possible contributory mechanism (46). In addition, receptors for GIP have been identified on many tissues, including islet cells, stomach, adrenal cortex, brain, bone, endothelial cells, adipocytes, and muscle (47). The presence of GIP-R on adipocytes is particularly interesting because there is increasing awareness that GIP has important effects on lipid metabolism (9). These include enhancement of chylomicron clearance, lipoprotein lipase activity, fatty acid synthesis, and reduction of postprandial triglycerides (48). Furthermore, fatty acids represent a particularly powerful stimulus to K-cell hyperplasia and GIP secretion, as already noted in *ob/ob* mice (25,26,49).

Additional work is required to assess the direct effects of GIP on insulin sensitivity. However, it seems likely that

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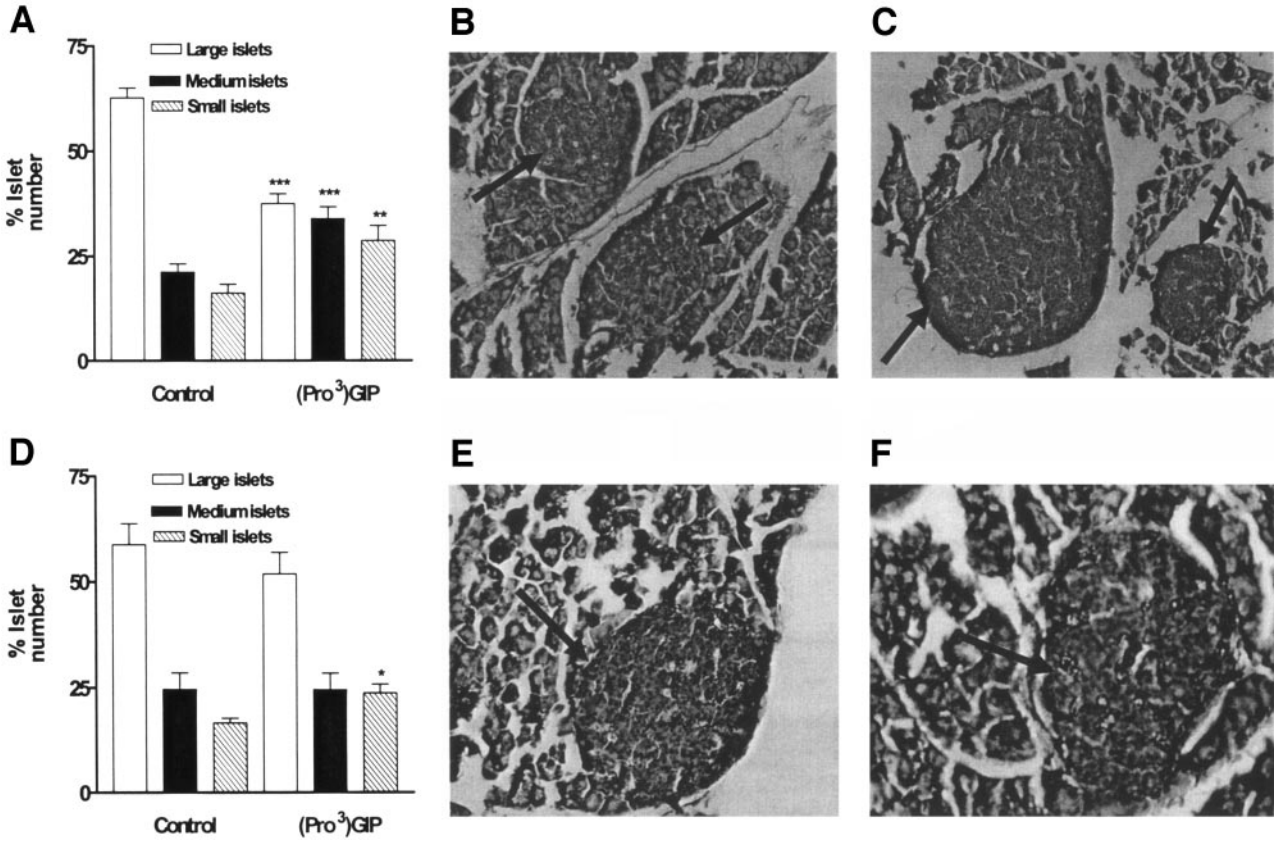

FIG. 8. Effects of daily (Pro^3)GIP administration on islet size and morphology in *ob/ob* mice. Parameters were measured after daily treatment with (Pro^3)GIP (25 nmol/kg body wt/day) or saline for 11 days (*A*) and 9 days after cessation of treatment. *D*: Proportion of islets classified as large diameter (>0.15 mm), medium diameter (0.1–0.15 mm), and small diameter (<0.1 mm) are shown. Values are means ± SE for eight mice. *B*, *C*, *E*, and *F*: Representative images (magnification ×40) of pancreata stained for insulin after 11 days treatment with (Pro^3)GIP (*B*) or saline (*C*); *E* and *F* represent corresponding images 9 days after cessation of treatment. The arrows indicate islets.

GIP-R antagonists may, in addition to enhancing insulin sensitivity through depression of hyperinsulinemia and consequent upregulation of insulin receptors, directly improve insulin action in both the absence and presence of decreased adipocyte lipid accumulation. Clearly, studies for much longer than the present 11-day period are required to evaluate the antiobesity potential of GIP-R antagonists as suggested by the results in genetic GIP-R knockout *ob/ob* mice reported by Miyawaki et al. (29).

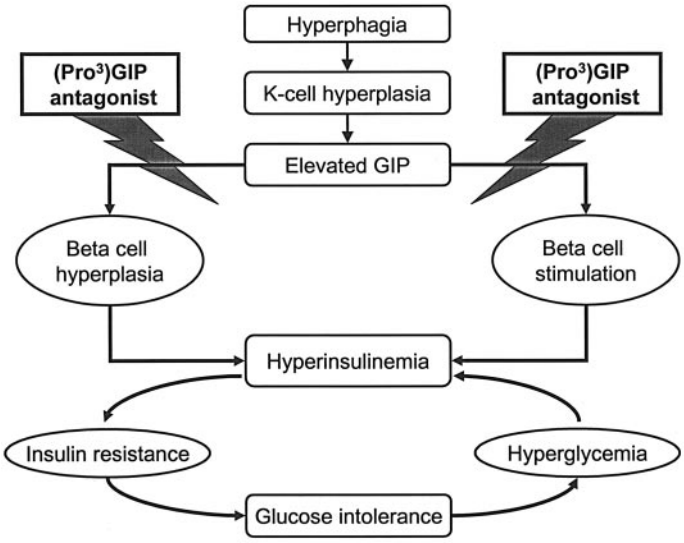

FIG. 9. Scheme illustrating how the GIP-R antagonist, (Pro^3)GIP, counters β-cell hyperplasia, hyperinsulinemia, and insulin resistance leading to improved glucose intolerance and diabetes control. Possible longer-term direct actions of GIP on adipocyte function and fat stores suggested by studies in GIP-R knockout *ob/ob* mice have been omitted.

In conclusion, the present study has demonstrated for the first time that daily administration of the GIP-R antagonist, (Pro^3)GIP, improves glucose tolerance and ameliorates insulin resistance and abnormalities of islet structure and function in *ob/ob* mice. Notably, these effects were reversed by discontinuation of (Pro^3)GIP for 9 days. Freedom from any obvious side effects is also in accordance with earlier observations in normal mice (42) and mice genetically engineered with life-long GIP-R deficiency (29). The present observations point to a cardinal role of endogenous GIP in the pathogenesis of obese insulin-resistant diabetes. More importantly, the data indicate that GIP-R antagonists, such as (Pro^3)GIP, might provide a novel, physiological, and effective means to treat obese type 2 diabetes through the alleviation of insulin resistance.

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This study is dedicated to the memory of Professor Keith D. Buchanan, friend and pioneer in the field of enteroinsular hormones.

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