

A novel pathway for regulation of glucose-dependent insulintropic polypeptide receptor expression in β -cells

Francis C. Lynn, Stephen A. Thompson, J. Andrew Pospisilik, Jan A. Ehses, Simon A. Hinke, Nathalie Pamir, Christopher H. S. McIntosh, and Raymond A. Pederson

Department of Physiology, University of British Columbia, Vancouver Canada, V6T 1Z3

Corresponding author: R. A. Pederson, Department of Physiology, Faculty of Medicine, University of British Columbia, 2146 Health Sciences Mall, Vancouver, British Columbia, Canada V6T 1Z3. E-mail: pederson@interchange.ubc.ca

ABSTRACT

Glucose-dependent insulintropic polypeptide (GIP) is secreted postprandially and acts in concert with glucose to stimulate insulin secretion from the pancreas. Here, we describe a novel pathway for the regulation of GIP receptor (GIPR) expression within clonal β -cell lines, pancreatic islets, and *in vivo*. High (25 mM) glucose was able to significantly reduce GIPR mRNA levels in INS(832/13) cells after only 6 h. In contrast, palmitic acid (2 mM) and WY 14643 (100 μ M) stimulated approximate doublings of GIPR expression in INS(832/13) cells under low (5.5 mM), but not high (25 mM), glucose conditions, suggesting that fat can regulate GIPR expression via PPAR α in a glucose-dependent manner. Both MK-886, an antagonist of PPAR α , and a dominant negative form of PPAR α transfected into INS(832/13) cells caused a significant reduction in GIPR expression in low, but not high, glucose conditions. Finally, in hyperglycemic clamped rats, there was a 70% reduction in GIPR expression in the islets and a 71% reduction in GIP-stimulated insulin secretion from the perfused pancreas. Thus, evidence is presented that the GIPR is controlled at normoglycemia by the fatty acid load on the islet; however, when exposed to hyperglycemic conditions, the GIPR is down-regulated, which may contribute to the decreased responsiveness to GIP that is observed in type 2 diabetes.

Key words: PPAR α • type 2 diabetes • Zucker rats • INS(832/13) cells • gastric inhibitory polypeptide

Postprandial insulin secretion is controlled by the gut-derived peptide hormones known as incretins. Glucose-dependent insulintropic polypeptide (GIP) and the proglucagon gene products glucagon-like peptide-1-(7-37) (GLP-1) and GLP-1-(7-36)-amide are the major incretins that act via this endocrine system (1). GIP stimulates pancreatic β -cell insulin secretion by binding to a serpentine, seven transmembrane, G protein-coupled receptor and subsequently activating adenylyl cyclase, phospholipase A₂ (PLA₂), and extracellular regulated kinases (ERK, MAP) as well as changing cellular ion fluxes (2–7).

Knockout mouse studies have demonstrated that both the GIP receptor (GIPR) and the GLP-1 receptor are integral to the release of insulin from the pancreas following a meal. Both GIPR and GLP-1 receptor null mice displayed compromised insulin secretion and therefore exhibited poor glucose tolerance to an oral glucose load (8, 9). Furthermore, *in vivo* administration of exendin (9–39) and GIP (7–30) antagonists at the GLP-1 receptor and the GIPR, respectively, decreased glucose tolerance to an oral glucose load in rats (10–12). From these studies, it has been estimated that together GIP and GLP-1 could account for more than 50% of the postprandial insulin secretory response.

The major stimuli for GIP secretion from the gastrointestinal tract are carbohydrates and fatty acids (13). Thus, it follows that GIP may play a role in fat metabolism in the adipocyte as well as other cell types expressing its receptor. Our laboratory has demonstrated that GIP is lipolytic in differentiated 3T3-L1 cells in a cAMP-dependent manner (14). Furthermore, McIntosh et al. (14) suggested that this lipolytic activity of GIP could prime the β -cell for the ensuing meal by causing an increase in free fatty acids in the circulation. However, other groups have shown GIP to be lipogenic in rat adipose tissue (15, 16). The role of GIP in fat metabolism in other cell types is at present poorly defined.

The peroxisome-proliferator activated receptors (PPARs) are a family of nuclear transcription factors that are activated *in vivo* by fatty acids; binding of an activator of PPAR α stimulates heterodimerization with the retinoid X receptor and then translocation to the nucleus, where transcriptional regulation can occur (17). PPAR α is expressed in the β -cell and is activated by free fatty acids such as palmitate and oleate as well as synthetic fibrate drugs such as clofibrate and WY 14643 (18, 19). Furthermore, PPAR α has been demonstrated to tightly control expression of genes involved in fatty acid oxidation in the pancreatic β -cell, including up-regulation of acyl-CoA-synthetase and carnitine palmitoyl transferase-1 (20). In addition, it is believed that activation of PPAR α is the main pathway by which leptin stimulates lipolysis in the pancreatic β -cell, thereby, protecting the β -cell from lipotoxicity (21).

Recently, it has been demonstrated that GIP is ineffective at stimulating insulin secretion in type 2 diabetes and VDF animal model of type 2 diabetes probably because there is a decrease in the expression of the GIPR on the β -cell in the disease (22–24). However, GLP-1-stimulated insulin secretion remains normal or even augmented in type 2 diabetes as well as in the hyperglycemic, hyperlipidemic VDF rat animal model (22, 25). Until now, the mechanisms governing GIPR down-regulation have been unclear. However, here, it is demonstrated that GIPR expression is tightly regulated by glucose as well as fatty acids in clonal β -cells and in pancreatic islets, and we propose that these regulatory systems may act to prime and protect the β -cell from the metabolic stresses elicited by fat and glucose in normal as well as diabetic patients.

MATERIALS AND METHODS

Synthetic human GIP (shGIP) was purchased from Bachem California (Torrance, CA). All chemicals, of reagent- or molecular biology-grade, were from Sigma (Oakville, ON, Canada) or Fisher Scientific International (Pittsburgh, PA). All tissue culture disposables were from BD Falcon (San Jose, CA).

Culture of BRIN-D11 and INS(832/13) cells

BRIN-D11 cells were obtained from Dr. P. B. Flatt (University of Ulster, Belfast, N. Ireland), and INS(832/13) cells were obtained from Dr. C. B. Newgard (University of Texas) (26, 27). Cell lines were maintained in a humidified atmosphere containing 5% CO₂ at 37°C. Both cell lines were grown in RPMI-1640 medium containing 11 mM glucose, supplemented with 10% fetal bovine serum (FBS) (Cansera, Rexdale, ON), and penicillin/streptomycin. The media in which the INS(832/13) cells were grown was supplemented with 10 mM HEPES (pH 7.4), 1 mM sodium pyruvate, 2 mM glutamine, and 50 μM β-mercaptoethanol.

Transfection of INS(832/13) cells

The mPPAR α -G construct was obtained from Dr. E. F. Johnson (Scripps Research Institute, La Jolla, CA), and dominant negative PPAR α construct hPPAR α_{tr} was obtained from Dr. B. Staels (Institut Pasteur de Lille, France) (28, 29). Cells were seeded at 6×10^6 cells/plate in 10-cm dishes. After 2 days of growth or when the cells were 90% confluent, transfection was carried out using Lipofectamine 2000 (Invitrogen, Burlington, ON, Canada) following the manufacturer's protocol. The day after transfection, the cells were transferred to 12-well plates at a seeding density of 1×10^6 cells/well and then allowed to grow for 24 h before the media was replaced and the experiment was started. Transfection efficiencies were determined by cotransfection of pGFPN2 (Invitrogen). Typically, the transfection efficiency was 40%.

Isolation and measurement of GIPR mRNA by real-time reverse transcriptase-polymerase chain reaction (RT-PCR)

For mRNA experiments, cells were seeded into 12-well plates at a density of 1×10^6 cells/well in RPMI or INS(832/13) media containing 5.5 mM glucose. Cells were grown in these media for 24 h before media was changed and experimental agents were applied. These included 100 μM WY 14643, 10 μM MK-886 (Biomol Research Laboratories, Plymouth Meeting, PA), 5 μM H89, 100 μM PD 98059, 2 μM bisindolylmaleimide (Bis) (Calbiochem, La Jolla, CA), 100 nM wortmannin (RBI/Sigma, Natick, MA), 1 μM insulin, or 2 mM palmitate (Sigma). After a further 24 h incubation, RNA was isolated using 0.5 ml/well Trizol and the standard protocol supplied by the manufacturer (Invitrogen).

Islets were isolated from lean Zucker rats as previously described (22) and grown overnight in RPMI-1640 media containing 11 mM glucose, supplemented with 10% FBS (Cansera), penicillin/streptomycin, 10 mM HEPES (pH 7.4), 1 mM sodium pyruvate, and 2 mM glutamine. Groups of 50 islets were then stimulated for 8 h with either 2 mM palmitate or 100 μM WY 14643. Following stimulation, RNA was isolated by addition of 1 ml of Trizol.

RNA was then quantified using the fluorescent Ribogreen reagent (Molecular Probes, Eugene OR). Following RNA isolation and quantification, 125 ng of RNA were subjected to reverse transcription. Total RNA was reverse transcribed in a volume of 10 μl containing 0.5 mM deoxynucleotide triphosphates, 15 pmol gene-specific primer targeted at the carboxy terminus of

the rat GIPR (5'-GTT CTG GAG TAG AGG TCC GTG TA-3'), 100 U Superscript II RNase H⁻ Reverse Transcriptase (Invitrogen), 10 U RNase inhibitor (RNA Guard; Amersham-Pharmacia, Piscataway, NJ), 1 mM dithiothreitol, 50 mM Tris-HCl (pH 8.3), 75 mM KCl, and 3 mM MgCl₂. Following reverse transcription, 25 ng of rat islet tissue cDNA was used in the real-time PCR reaction to measure GIPR expression. The PCR reaction mix consisted of 1× TaqMan Buffer A (PE Applied Biosystems, Foster City, CA), 10 mM MgCl₂, 200 μM dATP, dCTP, dGTP, and 400 μM dUTP, 200 nM rat GIPR 5' forward primer (5'-CCG CGC TTT TCG TCA TCC-3'), 200 nM rat GIPR 3' reverse primer (5'-CCA CCA AAT GGC TTT GAC TT-3'), 200 nM GIPR probe colabeled with the fluorescent dyes FAM and TAMRA (5'-CCC AGC ACT GCG TGT TCT CGT ACA GG-3'), 0.01 U/μl AmpErase uracil N-glycosylase (UNG, PE-Applied Biosystems), and 0.025 U/μl of AmpliTaq Gold (PE Applied Biosystems). PCR reactions were carried out in triplicate in the PE Applied Biosystems 7700 sequence detection system. The reaction profile included a 10-min preincubation at 50°C to allow the UNG to degrade any uracil containing nucleic acids and a further 10 min incubation at 94°C to activate the AmpliTaq Gold. Following these preincubations, a two-step PCR protocol was carried out, which included a denaturation step at 94°C for 15 s followed by a 1-min annealing/extension step at 60°C. Fluorescence was measured during the annealing/extension steps over 40 cycles and used to calculate a cycle threshold (Ct), that is, the point at which the reaction is in the exponential phase and is detectable by the hardware. All reactions followed the typical sigmoidal reaction profile, and a GIPR mRNA standard curve was used to determine amplicon abundance.

Glyceraldehyde phosphate dehydrogenase (GAPDH) was used as an internal control in earlier experiments because there was not an easy accurate way of determining RNA concentrations. When the Ribogreen reagent became available, we determined that it was more accurate to normalize to total RNA (30). For these GAPDH experiments, a commercially available probe and primer set was used (PE Applied Biosystems) for the PCR of 2.5 ng of cDNA; the reverse transcriptions for these experiments included 75 pmol of random hexamers.

RNA degradation and half-life

These studies were carried out by applying actinomycin D (5 μg/ml) to cells at various times following the beginning of the experiment and then measuring the amount of GIPR mRNA remaining using real-time RT-PCR (19).

Iodination of GIP and saturation binding experiments

As previously described, synthetic porcine GIP (5 μg) was iodinated by the chloramine-T method, and the ¹²⁵I-GIP was purified using reverse-phase HPLC to a specific activity of 350 μCi/μg (31). Aliquots of the tracer were lyophilized and stored at -20°C until needed. Cells were seeded into 24-well plates at a density of 5 × 10⁵ cells/well in 5.5 mM glucose containing medium. Following 24 h of culture, the medium was changed and experimental conditions were applied. After a further 24 h of culture, the cells were washed twice with ice-cold Krebs-Ringer bicarbonate buffer supplemented with 0.2 % BSA and 10 mM HEPES (KRBH). The saturation binding experiment was carried out at 4°C in KRBH containing 5.5 mM glucose and 1% Trasylol (aprotinin: Bayer, Etobicoke, ON, Canada) and varying amounts of radiolabeled ¹²⁵I-GIP (12.5–

112 fmol). Cells were washed twice with ice-cold KRBH, and radioactivity bound to cells was measured using a γ -counter. Nonspecific binding was defined as that measured in the presence of 1 μ M unlabeled shGIP. All binding data are expressed as specific binding of 125 I-GIP to cells.

Hyperglycemic clamp studies

Lean, 16-wk-old Zucker rats were anesthetized with an i.p. injection of sodium pentobarbital (65 mg/kg) (Somnotol; MTC Pharmaceuticals, Cambridge, ON, Canada). The right jugular vein was then exposed and cannulated with heparinized polyethylene tubing (PE50, Becton-Dickinson, Sparks, MD). Blood glucose measurements were taken every 10 min using a hand-held blood glucose meter (SureStep, Lifescan, Burnaby BC, Canada). Fifty percent glucose or saline was infused (0.5–3 ml/h) via the cannula by using an infusion pump (Harvard Apparatus, South Natick, MA), and the infusion rate was adjusted to maintain blood glucose levels of 5.5, 10, or 25 mM. Following 6 h of glucose clamping, the islets were isolated and GIPR mRNA levels were determined as described using real-time RT-PCR (22).

Pancreatic perfusions of high glucose clamped rats

Circulating glucose concentrations in rats were clamped as before; however, following 6 h of clamping, the pancreases of the rats were perfused as previously described with 25 pM human GIP (32). Samples were collected every minute, and insulin secretion was determined using radioimmunoassay as previously described (33).

Data analysis

All data are expressed as mean \pm SE, with the sample size indicated in the appropriate Figure legend. Two-tailed ANOVA and Tukey post hoc tests were performed, where $P \leq 0.05$ was considered statistically significant. Area under the curve was determined using curve analysis software (Graphpad, Prism, San Diego, CA).

RESULTS

Effects of glucose on GIPR mRNA expression in INS(832/13) cells

Glucose strongly down-regulated expression of GIPR mRNA in both a time- and a concentration-dependent manner ([Fig. 1A](#) and [1B](#)). As illustrated in [Figure 1B](#), there was a significant decrease in GIPR mRNA at glucose concentrations of 11 mM and above when compared with zero glucose conditions, with expression levels under the 25 mM condition decreased to 30% of that seen under zero glucose conditions. Furthermore, this decrease in receptor level occurred rapidly, with a significant difference being observed at 6 h following exposure to the 25 mM glucose conditions ([Fig. 1A](#)). No further decrease in receptor level was observed following the 18-h time point (cf. [Fig. 1A](#) and [1B](#)), at which time GIPR expression was reduced to 28% of the basal level. Culture of cells longer than 24 h in 25 mM glucose does not allow the cells to desensitize to the high glucose conditions, and the expression of GIPR mRNA remained at \sim 30% of the basal level (data not shown). Saturation binding analyses ([Fig. 2A](#))

showed a marked, statistically significant decrease in the amount of GIPR expressed on the cell surface of the INS(832/13) cells grown in high glucose conditions. The number of GIPR binding sites per cell grown at 5.5 mM glucose was 1930 ± 200 , whereas the number of GIPR binding sites per cell grown at 25 mM was 910 ± 130 . In addition, the dissociation constant (K_d) for GIPR was the same under both conditions, with K_d values of 86.00 ± 27 and 85.33 ± 29 fmol in 5.5 mM and 25 mM glucose, respectively. This indicates that the kinetics of binding were identical under both high and low glucose conditions.

The down-regulation of the GIPR mRNA was not due to a reduced half-life of GIPR mRNA. The half-lives of the mRNA encoding the GIPR were not statistically different at 5.5 and 25 mM glucose ([Fig. 3](#)). Thus, it does not appear that high glucose affected the RNA degradation pathway, and it is likely that there was a decrease in GIPR mRNA synthesis as a result of high glucose levels.

In an effort to determine how the down-regulation of the GIPR mRNA was occurring, we cultured INS(832/13) cells in the presence of various inhibitors of cell growth and proliferation (shown in [Fig. 4](#)). We did not see a reversal of the effects of 25 mM glucose in any of the conditions that we used. However, we observed that both wortmannin, a PI-3 kinase inhibitor, and H89, a PKA inhibitor, significantly increased GIPR mRNA levels above basal. Furthermore, we used insulin to ensure that high insulin levels were not contributing to the down-regulation of the GIPR, because high insulin levels occur during incubation of these cells in high glucose. As seen in [Figure 4](#), insulin increased GIPR expression and therefore was not contributing to the glucose-induced down-regulation. Neither Bis, a nonspecific PKC inhibitor, nor PD 98059, an MEK inhibitor, had any significant effect on GIPR expression at either glucose concentration ([Fig. 4](#)).

The effect of free fatty acids and PPAR α activation on GIPR expression in islets and in BRIN-D11 and INS(832/13) cells

Before acquiring the recently developed INS(832/13) cell line, we carried out initial experiments in BRIN-D11 β -cells. As shown in [Figure 5A](#), incubation of the BRIN-D11 cells in both 2 mM palmitate and WY 14643 produced significant increases in GIPR levels. Both stimuli produced an approximate threefold increase in GIPR expression in 5.5 mM glucose conditions. In addition, incubation of these cells in a medium containing a high fatty acid concentration up-regulated the GIPR expression at the cell surface as determined by saturation binding analyses. In fact, WY 14643 and palmitate both markedly increase cell surface GIPR expression in the BRIN-D11 cells (data not shown).

WY 14643 and palmitate also significantly increased GIPR mRNA levels in islets isolated from lean Zucker rats as seen in [Figure 5B](#). Palmitate was a stronger stimulant of receptor transcription in islets, producing an 11-fold increase in GIPR mRNA expression, whereas 100 μ M WY 14643 caused a 7-fold increase in receptor expression.

Fatty acids were also capable of increasing GIPR expression in the INS(832/13) cells. [Figure 6](#) shows that in the presence of 5.5 mM glucose, 2 mM palmitate significantly increased receptor

mRNA levels. [Figure 7](#) illustrates that the PPAR α activator, WY 14643, can increase receptor expression in INS(832/13) cells transfected with the mPPAR α -G form of the transcription factor, a mutant (G282E) form of PPAR α with low intrinsic transactivation properties but a higher affinity for WY 14643 and other fibrates than the wild-type form. Thus, both fatty acids and activation of PPAR α were able to up-regulate GIPR expression in the INS(832/13) cells.

As with the glucose studies, incubation of INS(832/13) cells in high fat or WY 14643 ([Fig. 3](#)) did not affect the degradation of GIPR mRNA. The half-lives of the GIPR mRNA in the cells that were grown in high fat (data not shown) and under control conditions were both \sim 30 min. Therefore, the increase in GIPR mRNA levels is probably a result of an increase in the transcription of GIPR mRNA.

In addition, stimulation of INS(832/13) cells with both WY 14643 and with 2 mM palmitate was able to increase cell surface GIPR expression approximately threefold ([Fig. 2B](#)). Therefore, as in the case of glucose-stimulated down-regulation of cell surface GIPR expression ([Fig. 2A](#)), induction of GIPR mRNA expression is directly linked to an increase in cell surface expression.

The interaction between fat and glucose and the effect on GIPR expression.

Recently, Roduit et al. showed that glucose induced down-regulation of PPAR α in INS(832/13) cells (19). We hypothesized that if GIPR expression was under the control of PPAR α , then glucose may result in down-regulation of the GIPR via a decrease in the ability of PPAR α to stimulate or maintain the basal level of expression. To test this hypothesis, we first incubated INS(832/13) cells in the presence of 2 mM palmitate in varying glucose concentrations. [Figure 6](#) shows that at glucose concentrations >8 mM, palmitate had no effect on GIPR expression. Furthermore, at high glucose levels (25 mM), fatty acids were unable to even maintain receptor levels at those seen basally and a significant decrease from basal level was observed. Furthermore, the PPAR α antagonist, MK-886 (34), caused a small decrease in GIPR expression at low glucose levels; however, it had no effect at levels >8 mM glucose ([Fig. 8](#)).

Finally, transfection of INS(832/13) cells with a high affinity form of PPAR α (mPPAR α -G) increased GIPR mRNA levels to 1.7 times basal levels in the presence of WY 14643 ([Fig. 7](#)). Transfection of INS(832/13) cells with a dominant negative form of PPAR α (28) caused a significant decrease in the expression of the GIPR to levels obtained with 5.5 mM glucose but had no effect at 25 mM glucose. Taken together, these data strongly suggest that PPAR α is able to maintain GIPR mRNA levels at low glucose but is ineffective at higher glucose levels.

Glucose-induced down-regulation of the GIPR in hyperglycemic clamped rats

Hyperglycemic clamps were performed on lean Zucker rats to determine whether high glucose was able to down-regulate GIPR expression *in vivo*. [Figure 9A](#) demonstrates that rats clamped at 25 mM glucose had only $33 \pm 7\%$ the GIPR mRNA level seen in 5.5 mM clamped animals. Those animals that were glucose clamped at 10 mM also showed a significant reduction in GIPR expression to $40 \pm 15\%$ of those seen in 5.5 mM clamped animals.

Concomitant with reduced GIPR expression, there was a reduction in GIP-stimulated insulin secretion from the perfused pancreas of animals that were clamped at 25 mM (Fig. 9B). This is reflected as a 71% reduction in the area under the curve in the treated animals as seen in the inset of Figure 9B. This indicates that the decrease in receptor levels could lead to a decreased functional ability of GIP to stimulate insulin secretion.

DISCUSSION

In type 2 diabetes, there is a marked reduction in the insulinotropic potency of GIP. Recently, we have shown that the cause of this reduction in potency may be a decreased GIPR expression on the β -cells in the Vancouver diabetic fatty Zucker rat (VDF) model of type 2 diabetes (22). However, currently there are no data to suggest the mechanisms by which GIPR down-regulation occurs in type 2 diabetic patients or in animal models of the disease. Here, we demonstrate that elevated glucose is able to significantly abrogate GIPR expression in both cells and animal models and that this effect is not reversed by blocking any of the common cell growth and proliferation pathways. We also demonstrate a novel pathway for stimulation of GIPR expression at normal glucose levels through fat-stimulated PPAR α activation, which is unable to reverse the GIPR down-regulation seen at higher glucose levels.

Recently, Roduit et al. and Laybutt et al. demonstrated that high glucose caused down-regulation of PPAR α in INS(832/13) cells and in pancreatectomized rats, respectively (19, 35). The time course for down-regulation of PPAR α in 20 mM glucose was almost identical to that observed for the down-regulation of GIPR by high glucose reported here. Where we saw a significant reduction in GIPR expression after only 6 h in high glucose (Fig. 1A), Roduit et al. saw a significant and total ablation of PPAR α expression at 6 h (19). Their study also demonstrated that down-regulation of PPAR α led to a decreased expression of the mRNA for uncoupling protein 2 (UCP2), carnitine palmitoyltransferase 1 (CPT 1), and acyl-CoA oxidase, genes that all have well-defined PPAR response elements in their promoters. Therefore, down-regulation of PPAR α by glucose can cause a down-regulation of genes normally controlled by this nuclear transcription factor. Finally, their study demonstrated that 0.4 mM oleate had no effect on PPAR α expression in INS(832/13) cells in the presence of either high or low glucose levels. Thus, the observation that free fatty acids (FFA) were unable to increase GIPR mRNA levels under high glucose conditions was not a result of down-regulation of PPAR α by fatty acids but rather because PPAR α was down-regulated by elevated glucose.

To determine whether the down-regulatory effects of glucose on GIPR expression could be attributed to the action of a common signal transduction pathway, we applied various inhibitors of these pathways to cells for 24 h. As can be seen in Figure 4, none of these inhibitors reversed the glucose-induced down-regulation of GIPR expression. However, we made several interesting observations. First, we observed that wortmannin, a PI-3K inhibitor; H89, a PKA inhibitor; and insulin all increased receptor expression. Insulin was included as a control because it was observed that under high glucose conditions, the amount of insulin in the media was 2.5 times that under basal conditions (data not shown) and it was suspected that insulin could be causing the down-regulation of the GIPR. However, insulin appeared to have the opposite effect. The apparent contradiction between the insulin and wortmannin data could be explained by a

desensitization of the insulin signaling pathway in these β -cells by a prolonged, potent stimulation with insulin (36, 37). Thus, we expect that long-term stimulation with insulin probably had much the same functional effect as stimulation with wortmannin. Interestingly, the mitogen-activated protein (MAP) kinase signaling module has been implicated in the activation of PPAR α , and we did see a small but not significant decrease in the expression of the GIPR at 5.5 mM glucose in the INS(832/13) cells that were incubated with the MEK inhibitor PD 98059. These data indicate that an actual decrease in PPAR α expression is probably more important than the activation (phosphorylation) state of PPAR α in the regulation of GIPR expression.

The control of GIPR expression by PPAR α is limited to low glucose conditions, at which point it stimulates an increase in expression. The physiological significance of this is obscure because at low glucose levels, GIP is not effective at stimulating insulin secretion; however, in the presence of 0 mM glucose, GIP is able to stimulate adenylyl cyclase, resulting in cAMP accumulation (38), as well as activate MAP kinase (J. A. Ehses, unpublished observations) and PLA $_2$ (2). Therefore, it is possible that GIP has functional roles in the β -cell in addition to insulin secretion. Intraduodenal fat is probably the most important stimulant of GIP secretion from the gut and therefore it follows that fat should be able to regulate GIPR expression. In this manner, stimulation by free fatty acids or long-chain acyl-CoA esters derived from either the adipocyte during the interdigestive period or early in the prandial process may ready the β -cell for the ensuing GIP stimulation. In addition, recent data from our laboratory (F. C. Lynn, unpublished data) shows that GIP stimulates fatty acid oxidation within the pancreatic β -cell, thus GIP may act to prime the β -cell with ATP, using intracellular fat stores. This would allow a more rapid glucose stimulation of insulin secretion.

When glucose levels are high, we see a dramatic and reproducible down-regulation of the GIPR *in vivo* and *in vitro* (Fig. 1, 2, 6–9). However, fat is no longer able to induce GIPR expression at high glucose levels. This also makes physiological sense if GIP is acting to cause fat oxidation within the β -cell in order to prime the insulin secretory pathways for the ensuing meal. It would thus be expected that when glucose levels are high, the β -cell would no longer have a need for GIP-stimulated oxidation of fatty acids. Therefore, expression of the GIPR is down-regulated and GIP becomes ineffective at high glucose levels. The down-regulation occurs extremely quickly, with a statistical difference seen after only 6 h in high glucose. This time course would allow GIP to have an incretin effect on the β -cell, but would limit its actions in prolonged hyperglycemia. In addition, our group has shown that in the short term GIPRs are quickly internalized in response to GIP, with a significant reduction in cell surface receptors occurring after only 10 min of exposure to GIP (38). Thus, within minutes of GIPR activation, bioactivity is probably governed by phosphorylation events, but in the hours following, GIPR activity is probably controlled by the level of expression of the receptor at the cell surface. Accordingly, due to the prolonged elevation of blood glucose in type 2 diabetic individuals, GIPR levels are decreased.

Interestingly, this data fits with the hypothesis put forward by Prentki et al. in that glucose seems to positively regulate expression of genes involved in its metabolism and negatively regulate genes involved in metabolism of other fuels (39). Accordingly, free fatty or nonesterified fatty acids stimulate expression of genes involved in their metabolism such as CPT 1 (40). Thus,

GIPR expression seems to be regulated in a manner that is consistent with other metabolic genes within the β -cell. In addition, one pathway by which GIP could stimulate β -cell function and cytoprotection could be by decreasing fatty acid levels within the cell, thereby preventing lipotoxicity.

It is not known whether the action of PPAR α on GIPR expression is a direct effect or if it occurs via activation of other transcription factors. For example, Schinner et al. (41) recently demonstrated that activation of PPAR γ inhibits glucagon expression in the α -cell by inhibiting Pax6 transcriptional activity. The pancreatic β -cell also expresses Pax6, and it could conceivably interact with PPAR α to stimulate receptor expression. Further studies using the recently cloned GIPR promoter need to be carried out to determine the exact sequence elements that control the fatty acid-stimulated increase in receptor expression.

In conclusion, the current studies have demonstrated a novel pathway by which glucose and fat can control GIPR expression in both clonal β -cells and under *in vivo* conditions. Free fatty acids are able to bind to and activate PPAR α at low glucose conditions and stimulate GIPR transcription either directly or indirectly. However, at high glucose conditions, PPAR α itself is down-regulated and is no longer able to maintain basal GIPR expression. These results can account for the down-regulation of the GIPR that is observed in the hyperglycemic, hyperinsulinemic VDF model of type 2 diabetes and may underlie the decreased responsiveness of type 2 diabetic patients to GIP.

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Fig. 1

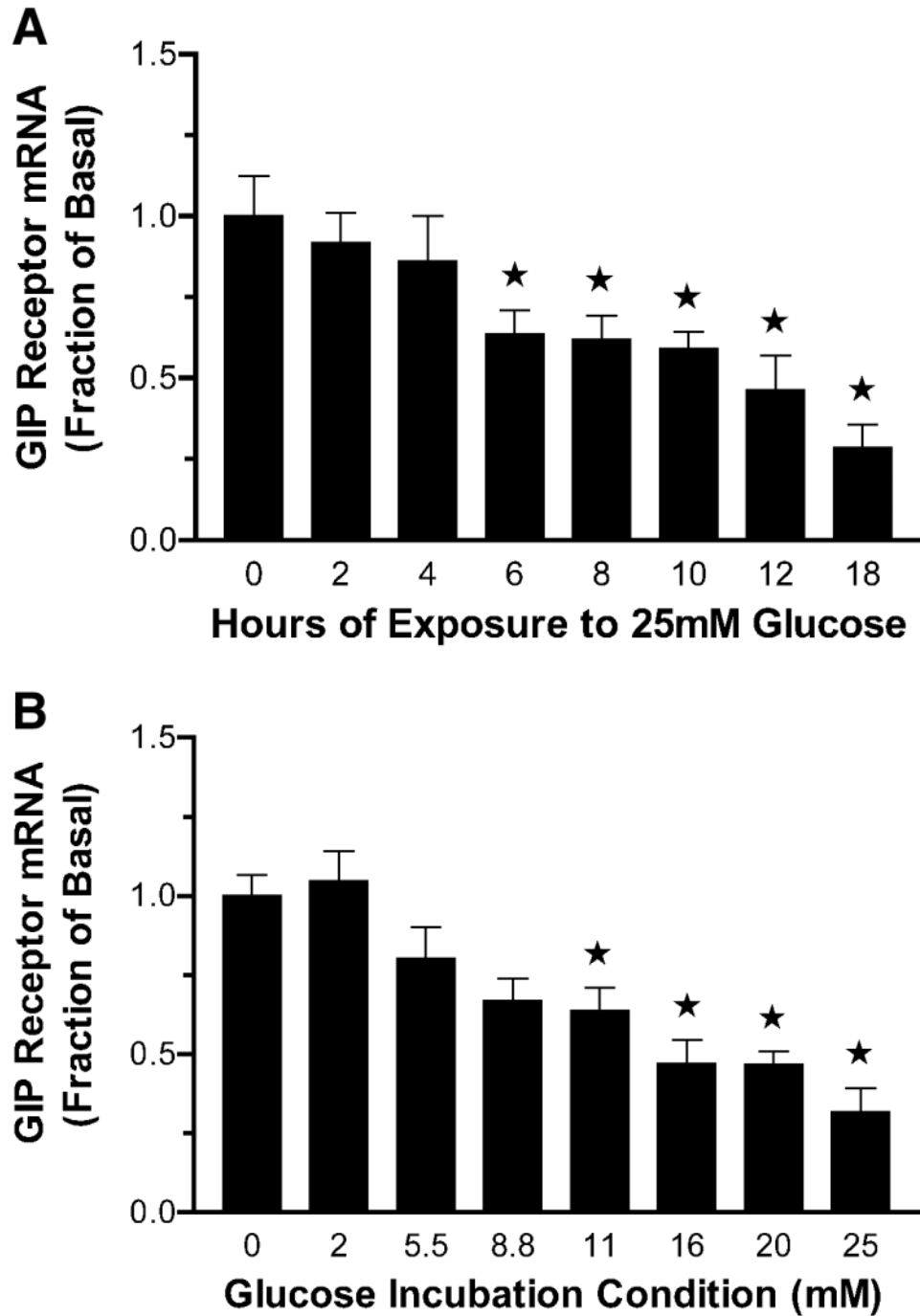


Figure 1. The effect of glucose on GIP receptor mRNA expression in INS(832/13) cells. A) Time course for glucose-induced GIP receptor down-regulation. Cells were incubated in regular media supplemented with 25 mM glucose for times varying between 0 and 24 h. **B)** Glucose dose response of GIP receptor mRNA down-regulation. Cells were incubated for 24 h in varying glucose concentrations between 0 and 25 mM. Following incubation, RNA was isolated and quantified using real-time RT-PCR as described in Materials and Methods. Data were normalized to the basal conditions: for A, this was 0 h; for B, it was 0 mM glucose. Asterisks indicate statistical significance compared with basal levels ($P < 0.05$, $n = 4$).

Fig. 2

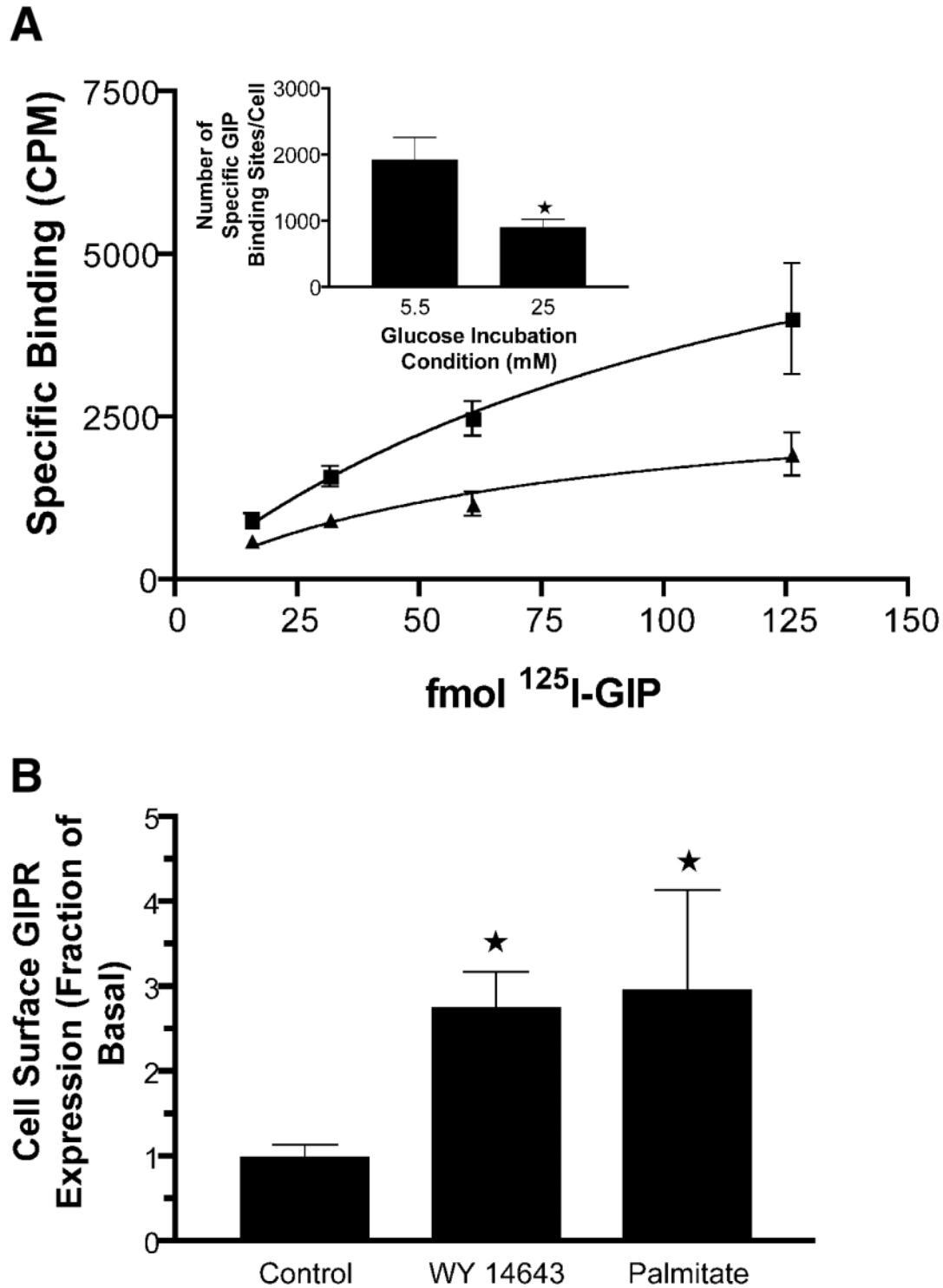


Figure 2. Saturation binding analyses of INS(832/13) cells treated with high glucose (A) or WY 14643 and 2 mM palmitate (B). Cells were incubated for 24 h in either 5.5 mM or 25 mM glucose (A) or 5.5 mM glucose with 100 μM WY 14643 or with 2 mM palmitate (B). Following incubation, varying amounts of ^{125}I -GIP were added to the cells and allowed to come to equilibration over 4 h at 4°C. Cells were then washed, and specific binding was calculated. Inset A: Number of cell surface receptors under both conditions over four separate experiments. B: Expressed as a fraction of basal (5.5 mM) cell surface receptors. * $P < 0.05$.

Fig. 3

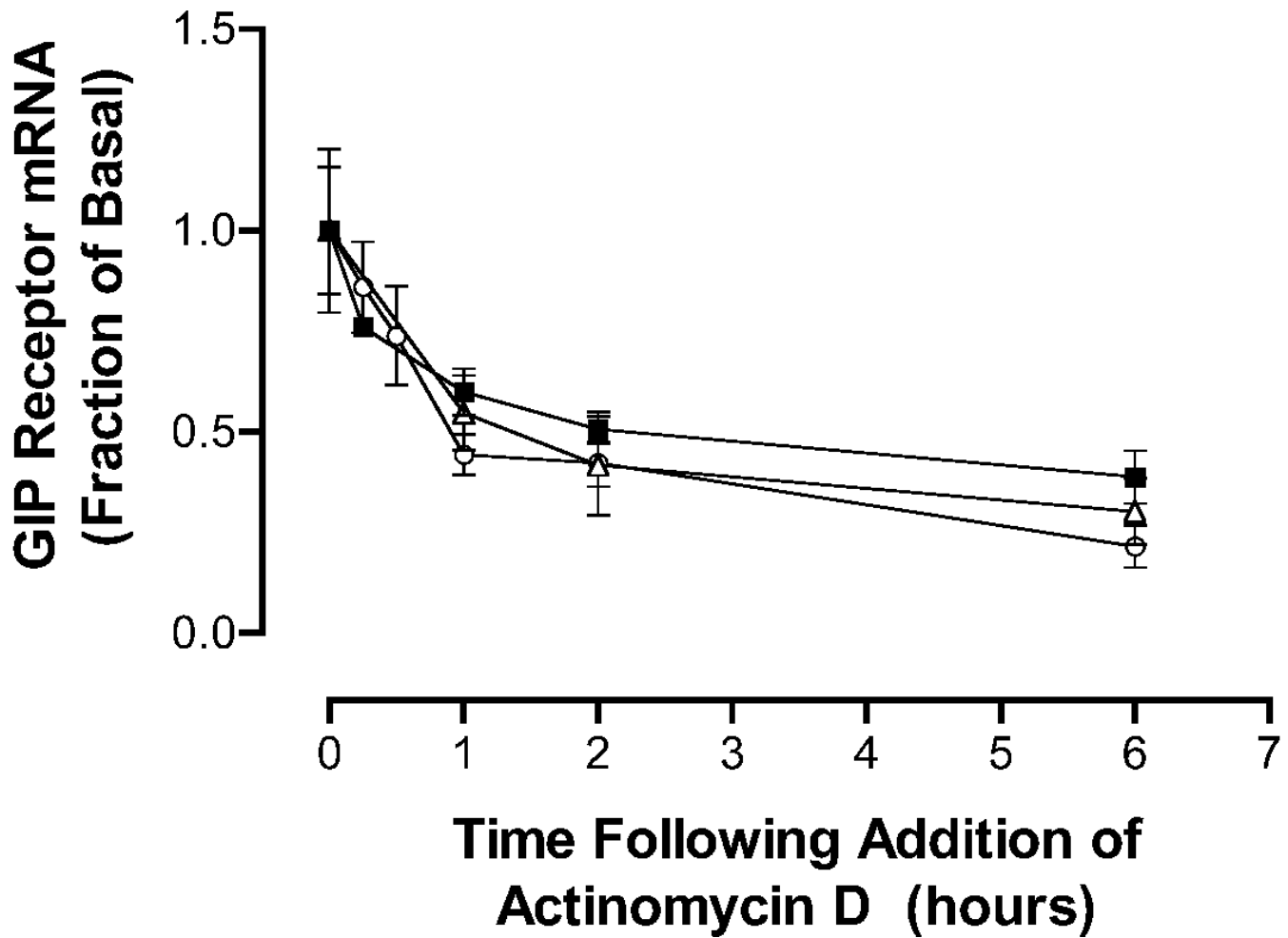


Figure 3. GIP receptor mRNA degradation curves in INS(832/13) cells. Cells were exposed to 5.5 mM glucose (■), 25 mM glucose (Δ), or WY 14643 (100 μM) (O) for 24 h before the addition of 5 μg/ml actinomycin D. Cells were then allowed to incubate with actinomycin D for varying times between 0 and 6 h before RNA was harvested and GIP receptor mRNA expression was assessed by real-time RT-PCR. Data are expressed as a fraction of that seen at basal conditions or before addition of actinomycin.

Fig. 4

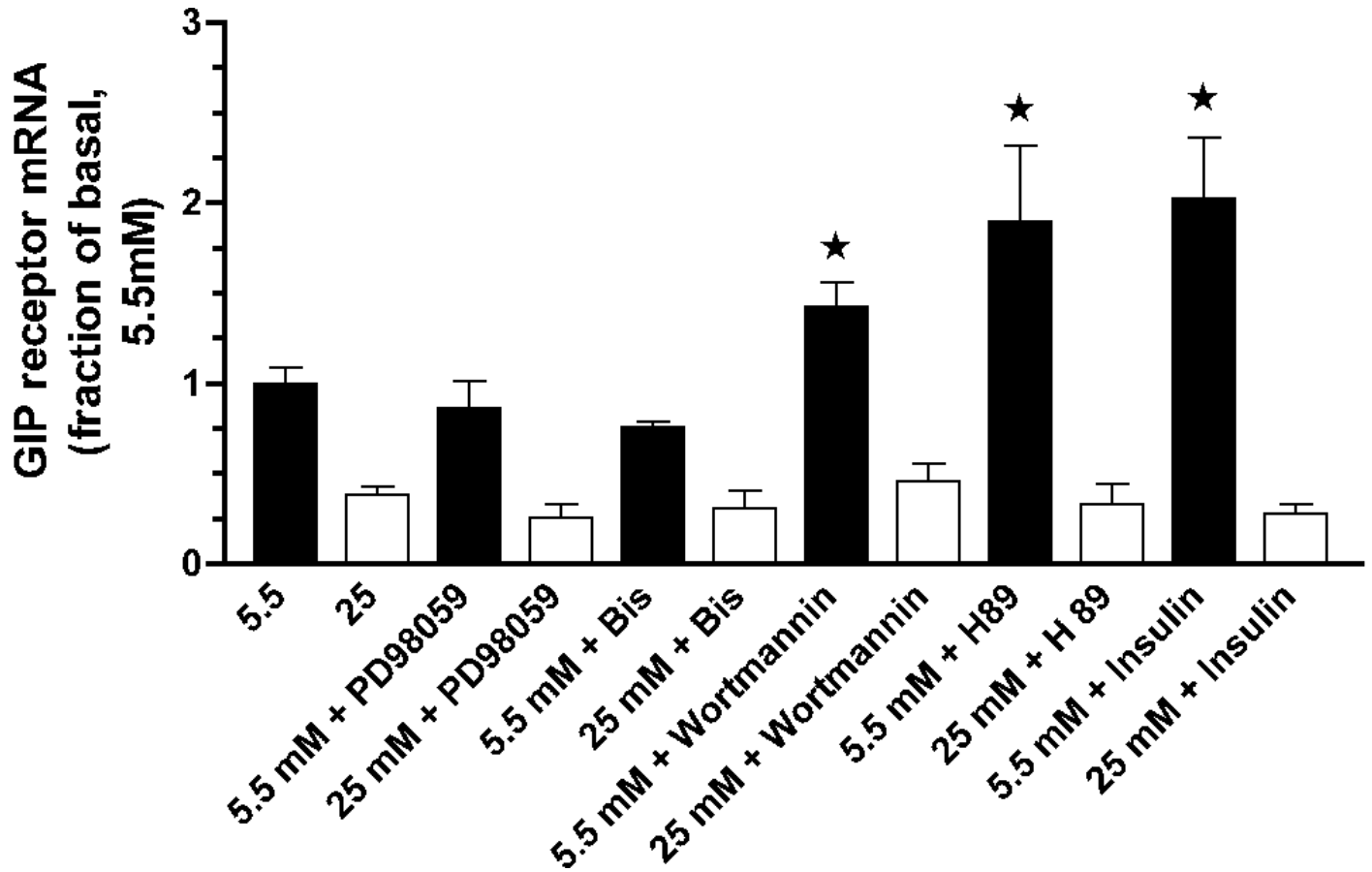


Figure 4. The effect of various inhibitors of cell growth and proliferation on glucose-induced GIP receptor mRNA down-regulation in INS(832/13) cells. Cells were grown for 24 h in either 5.5 or 25 mM glucose in the presence of inhibitors (5 μ M H89, 100 μ M PD 98059, 2 μ M B is 100 nM wortmannin, 1 μ M insulin) as described in Materials and Methods. Following this incubation period, RNA was harvested and GIP receptor mRNA levels were measured using real-time RT-PCR. Data are expressed as a fraction of basal (5.5 mM) conditions ($*P < 0.05$, $n=3$).

Fig. 5

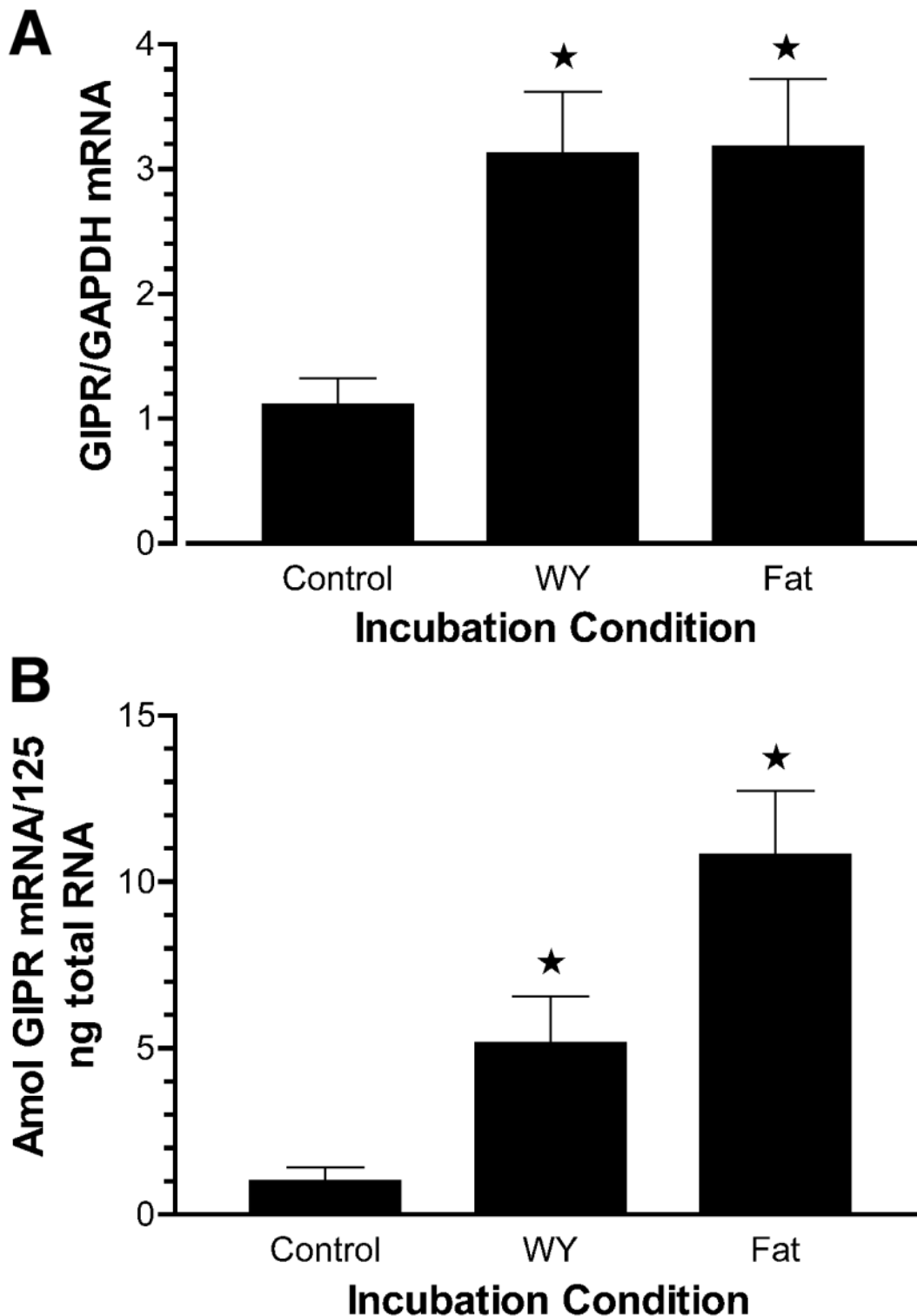


Figure 5. GIP receptor expression in BRIN-D11 cells (A) and islets (B) following incubation with PPAR α activator, 100 μ M WY 14643, or 2 mM palmitate. A) BRIN-D11 cells were cultured for 24 h in the presence of either WY 14643 or 2 mM palmitate (Fat). RNA was then isolated, and GIP receptor expression was quantified using real-time RT-PCR. GIP levels were normalized to GAPDH mRNA levels ($*P < 0.05$, $n = 3$). **B)** Islets were isolated from lean Zucker rats and then cultured overnight in 11 mM glucose. Following this recovery period, the islets were incubated at 5.5 mM glucose with either WY 14643 or 2 mM palmitate for 8 h before RNA was harvested. GIP receptor expression was determined by carrying out real-time PCR on total islet RNA (significance from control conditions, $*P < 0.05$, $n = 4$).

Fig. 6

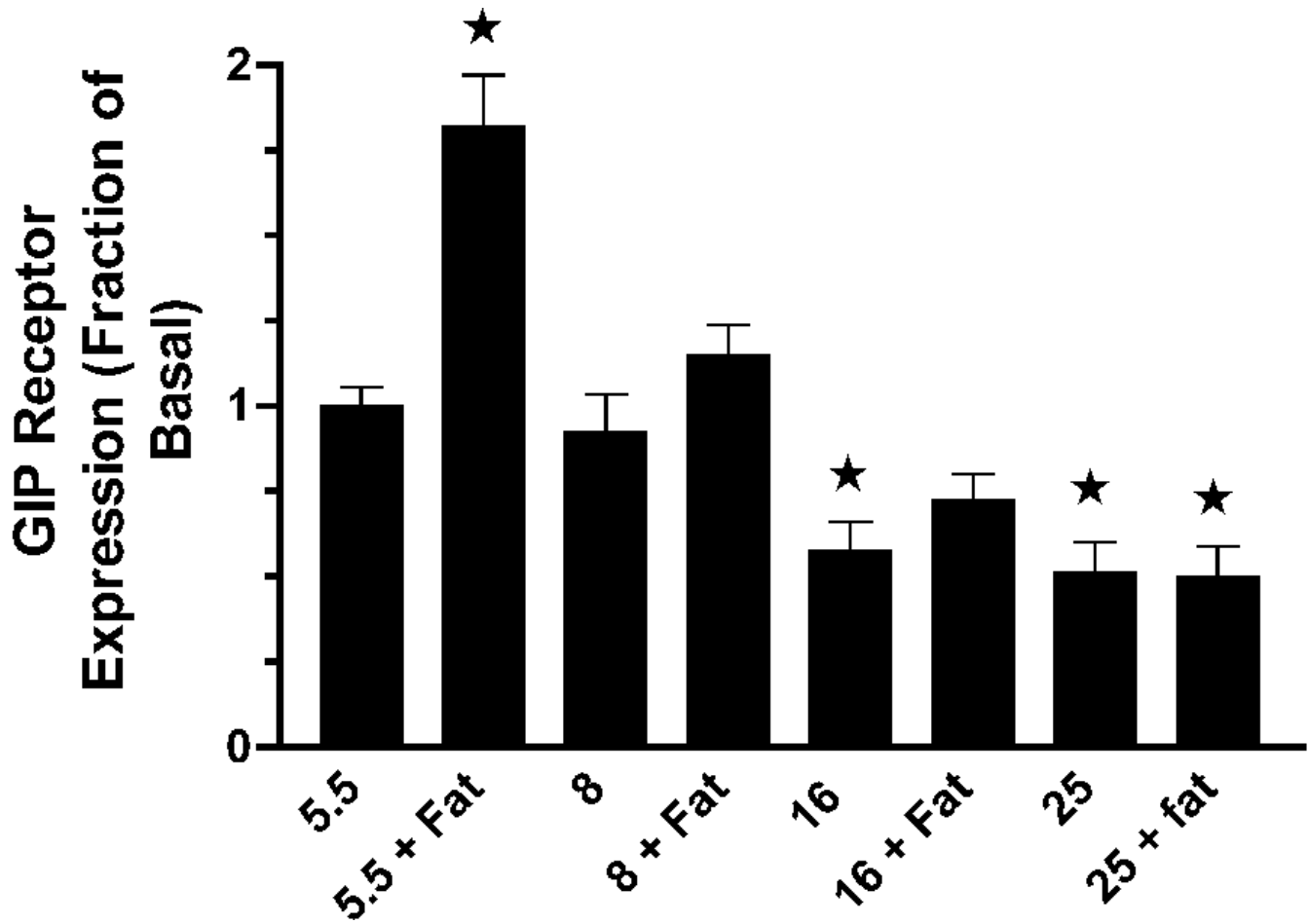


Figure 6. GIP receptor mRNA expression following culture of INS(832/13) cells for 24 h in various glucose concentrations with 2 mM palmitate. Cells were incubated overnight in 5.5, 8, 16, or 25 mM glucose in the presence or absence of 2 mM palmitate. (Fat). Following this incubation RNA was harvested and subjected to real-time PCR for quantification of GIP message (statistical significance compared with basal, 5.5 mM conditions, $P < 0.05$, $n = 4$).

Fig. 7

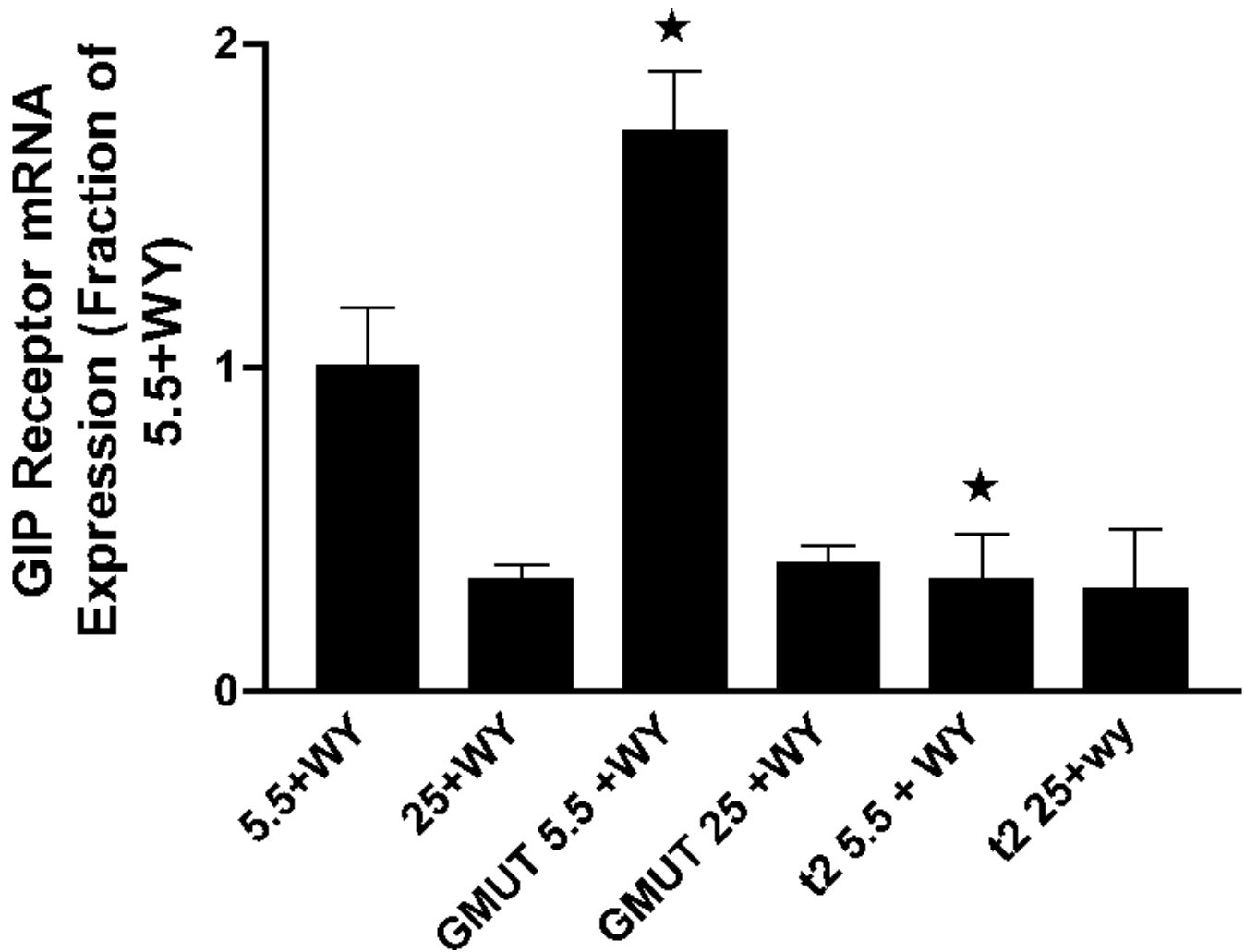


Figure 7. Effect of stimulating or blocking PPAR α activity in INS(832/13) cells. Cells were transfected with two mutant PPAR α isoforms: either the GMUT (mPPAR α -G) form, which has a increased affinity for WY 14643, or the hPPAR α_{tr} form, which is a dominant negative protein as described in Materials and Methods. Cells were then grown for 24 h in the presence of WY 14643 in either high (25 mM) or low (5.5 mM) glucose. RNA was harvested, and GIP receptor expression was quantified using real-time RT-PCR (statistical significance compared with 5.5 + WY 14643, * $P < 0.05$, $n = 3$).

Fig. 8

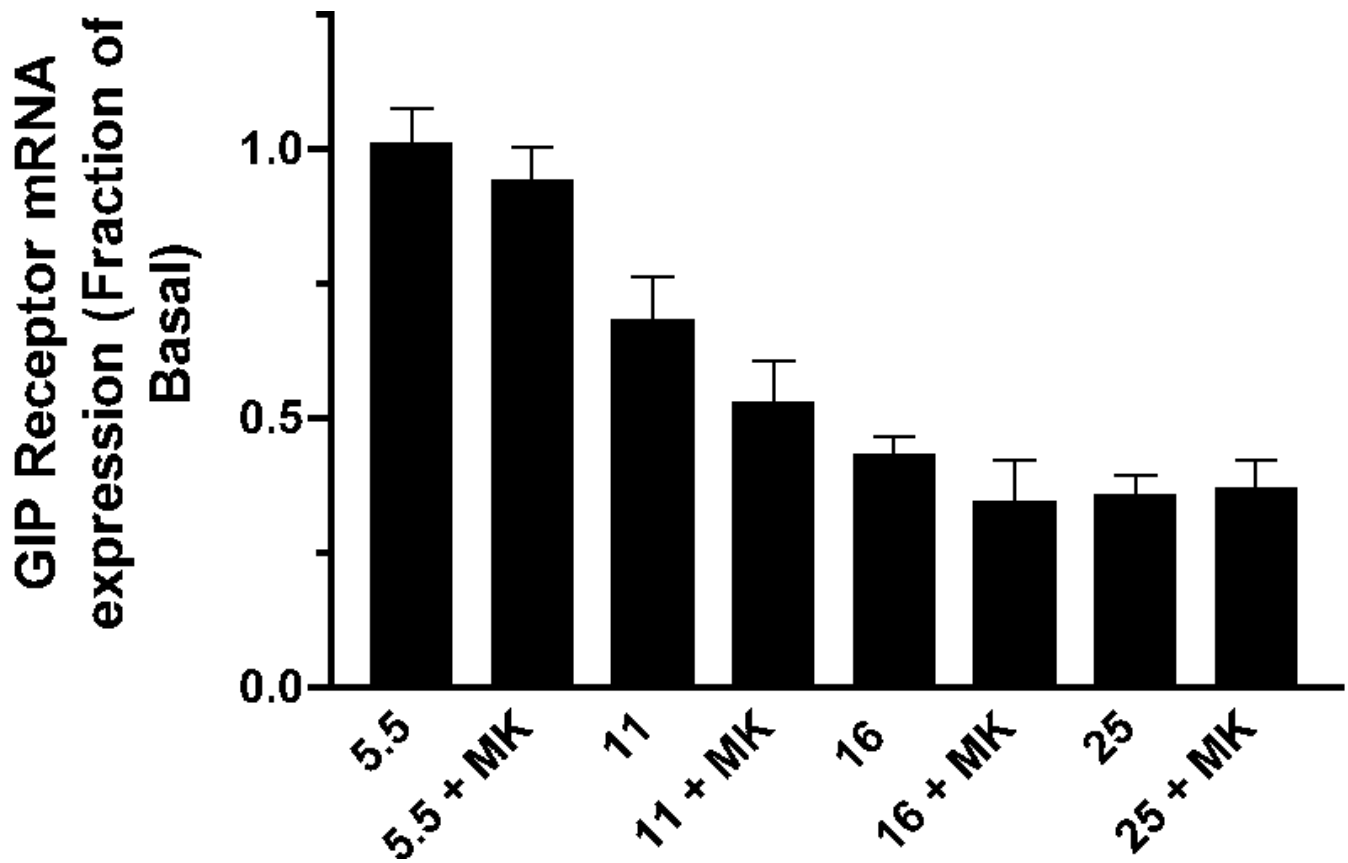


Figure 8. Effect of a specific PPAR α antagonist on glucose-induced GIP receptor down-regulation. Cells were grown in 5.5, 11, 16, or 25 mM glucose in the presence or absence of MK-886, a PPAR α antagonist, for 24 h. RNA was then harvested and subjected to real-time RT-PCR for analysis of GIP receptor expression. Data are expressed as a fraction of the 5.5 mM condition; $n=4$.

Fig. 9

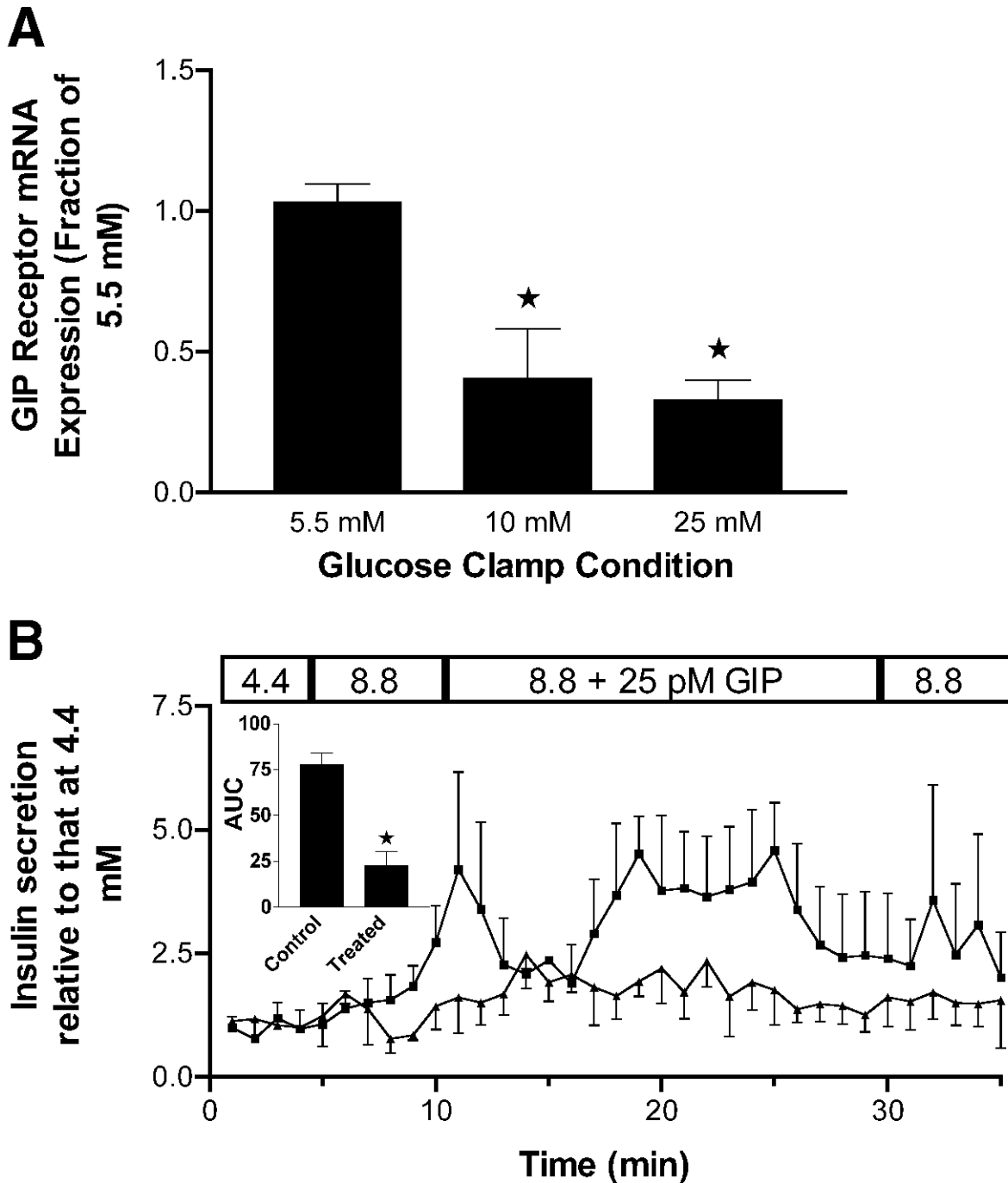


Figure 9. A) Effect of hyperglycemic clamp on GIP receptor expression in the islets of lean Zucker rats. Plasma glucose levels of anesthetized lean Zucker rats were clamped at 5.5, 10, or 25 mM glucose for 6 h. Islets were then harvested, and RNA was isolated for subsequent real-time RT-PCR. *Statistical significance compared with basal conditions. **B) Effect of hyperglycemic clamp on GIP-stimulated insulin release from the perfused lean Zucker rat pancreas.** Lean animals were clamped at 5.5 (■) or 25 (Δ) mM glucose for 6 h before pancreatic perfusion with the protocol outlined in Materials and Methods. Insulin secretion is expressed as a fraction of that seen in the average of the first 5 min of the perfusion. Area under the curve was determined using the trapezoidal method and plotted as a bar graph in the inset (statistical significance, * $P < 0.05$, $n = 3$).