

A novel pathway for regulation of glucose-dependent insulinotropic polypeptide (GIP) receptor expression in β cells¹

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SPECIFIC AIM

Glucose-dependent insulinotropic polypeptide (GIP) is secreted postprandially and acts in concert with glucose to stimulate insulin secretion from the pancreas. In type 2 diabetic patients and animal models of the disease where there is persistent hyperglycemia, the ability of GIP to stimulate insulin secretion is severely abrogated due to a decreased expression of GIP receptors on the pancreatic β cell. Here we set out to define the mechanisms by which GIP receptors (GIPR) down-regulation could occur in type 2 diabetes.

PRINCIPAL FINDINGS

1. Glucose down-regulates GIP receptor expression in a concentration- and time-dependent manner

Glucose (25 mM) was able to significantly reduce GIPR mRNA to $64 \pm 7\%$ and $28 \pm 6\%$ basal levels in INS(832/13) cells after 6 and 24 h, respectively. Furthermore, 24 h of incubation with 11 mM glucose caused a significant, $36 \pm 7\%$, reduction in GIPR expression in these cells. Saturation binding analyses showed a marked, statistically significant decrease in the amount of GIP receptor expressed on the cell surface of the INS(832/13) cells grown in high glucose conditions: 1930 ± 200 and 910 ± 130 binding sites per cell for cells grown respectively in 5.5 and 25 mM glucose.

Hyperglycemic clamps were performed on lean Zucker rats to determine whether high glucose was able to down-regulate GIPR expression in vivo. **Figure 1A** 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 clamped at 10 mM glucose, a realistic fasting blood glucose level for type 2 diabetes, also showed a significant reduction in GIP receptor expression to $40 \pm 15\%$ of that seen in 5.5 mM clamped animals (Fig. 1A). Concomitant with reduced GIPR expression, there was a reduction in GIP-stimulated insulin secretion from the perfused

pancreata of animals that were clamped at 25 mM (Fig. 1B). This is reflected as a $71 \pm 17\%$ reduction in the area under the curve in the treated animals as seen in the inset of Fig. 1B. This indicates that the decrease in receptor levels could lead to a decreased functional ability of GIP to stimulate insulin secretion in type 2 diabetes.

Down-regulation of GIPR mRNA was not due to a reduced half-life of the mRNA. Thus, it does not appear that high glucose affected the RNA degradation pathway, and it is likely there was a decrease in GIPR mRNA synthesis as a result of high glucose levels.

To determine the mechanism of GIPR down-regulation, INS(832/13) cells were cultured in the presence of various inhibitors of cell proliferation. There was no reversal of the effect of 25 mM glucose in any condition used (i.e., PI-3 kinase, PKA, PKC, or MEK inhibition). We used insulin to ensure that high insulin levels were not contributing to the down-regulation of the GIPR, as high insulin levels occur during incubation of these cells in high glucose. Insulin increased GIP receptor expression and therefore was not contributing to the glucose-induced down-regulation.

2. Free fatty acids and peroxisome-proliferator activated receptor (PPAR) α activators elevate GIP receptor expression at low but not at high glucose levels.

Palmitate (2 mM) was a strong stimulant of receptor transcription in isolated lean Zucker rat islets, producing an 11 ± 2 -fold increase in GIP receptor mRNA expression, whereas 100 μ M WY 14643 caused a 7 ± 1 -fold increase in receptor expression.

Figure 2 shows that in the presence of 5.5 mM

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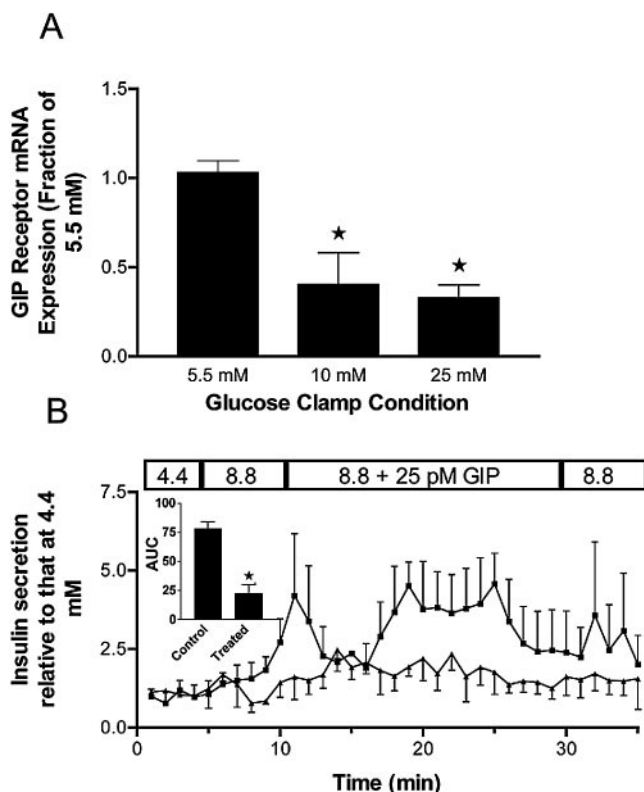


Figure 1. A) The effect of hyperglycemic clamping on GIP receptor expression in the islets of lean Zucker rats. Plasma glucose levels of anesthetized lean Zucker rats were clamped at either 5.5, 10, or 25 mM glucose for 6 h. Islets were then harvested and RNA was isolated for subsequent real-time RT-PCR. Asterisks indicate statistical significance compared to basal conditions. B) The 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. 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. Asterisks indicate statistical significance $P < 0.05$; $n = 3$.

glucose, 2 mM palmitate significantly increased receptor mRNA levels to 1.8 ± 0.2 -fold basal levels in the INS(832/13) cells. WY 14643 increased receptor expression to 1.7 x basal levels in INS(832/13) cells transfected with the mPPAR α -G form of the transcription factor. Palmitate and WY 14543 were both able to increase cell surface GIPR expression in these cells. Thus, fatty acids and activation of PPAR α were both able to up-regulate GIPR expression in INS(832/13) cells at the transcriptional level.

It was recently demonstrated that high glucose stimulates down-regulation of PPAR α in INS(832/13) cells and pancreatectomized rats. We hypothesized that if GIP receptor expression was under the control of PPAR α , then glucose may result in down-regulation of the GIP receptor via a decrease in the ability of PPAR α to stimulate or maintain the basal level of expression. To test this hypothesis, we incubated INS(832/13) cells in the presence of 2 mM palmitate (which is an

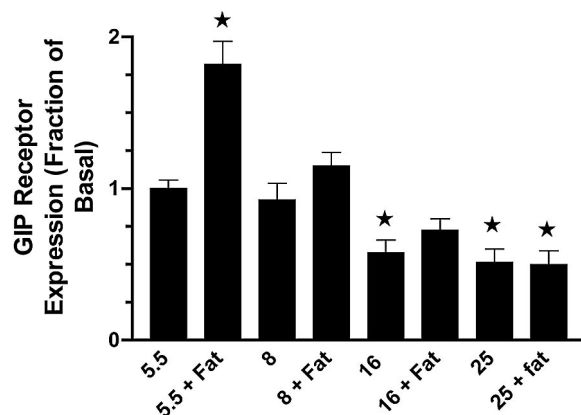


Figure 2. GIP receptor mRNA expression after culture of INS(832/13) cells for 24 h in various glucose concentrations with 2 mM palmitate. Cells were incubated overnight in 5.5, 8, 16, or 25 mM glucose in the presence or absence of 2 mM palmitate (Fat). RNA was harvested and subjected to real-time PCR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

endogenous activator of PPAR α) in varying glucose concentrations. Figure 2 shows that at glucose concentrations > 8 mM, palmitate had no effect on GIP

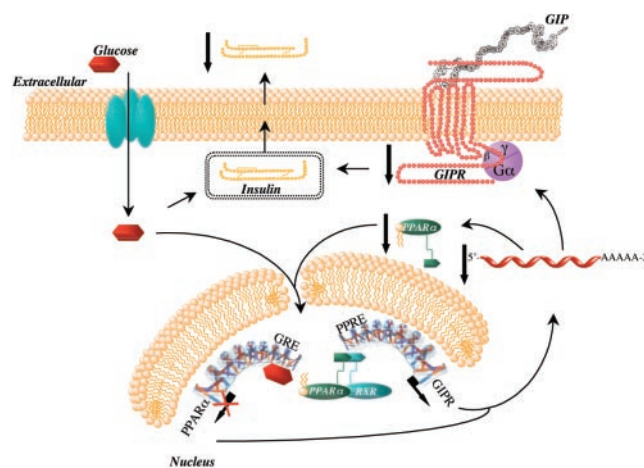


Figure 3. Glucose-dependent insulinotropic polypeptide (GIP) binds to its receptor (GIPR) activating adenylyl cyclase, thereby potentiating glucose-induced insulin secretion. Glucose crosses the β cell membrane via GLUT-2 transporter and is metabolized, which leads to an increase in the ATP/ADP ratio, depolarization of the β cell, and insulin secretion. Under fasting or low glucose conditions, free fatty acids bind to peroxisome proliferator-activated receptor α (PPAR α). PPAR α then heterodimerizes with the retinoid X receptor (RXR), translocates to the nucleus, and binds to a peroxisome proliferator response element (PPRE), thus stimulating GIPR transcription and leading to an increase in cell surface GIPR expression. However, high glucose inhibits (PPAR α) transcription via a response element (GRE) within the PPAR α promoter. This causes a reduction in PPAR α transcription and leads to a decrease in the PPAR α expression level. With a cellular reduction in PPAR α , it is no longer able to fully stimulate GIPR expression and its expression also falls. This reduction in GIPR expression causes a decreased insulin secretion in response to GIP from the β cell.

receptor expression. At high glucose levels (25 mM), fatty acids were unable to maintain receptor levels even at those seen basally, and a significant decrease from basal level was observed.

Transfection of INS(832/13) cells with a dominant negative form of PPAR α caused a significant decrease in expression of the GIP receptor to levels obtained with 5.5 mM glucose while having 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.

CONCLUSIONS AND SIGNIFICANCE

The control of GIP receptor expression by PPAR α is limited to low glucose conditions at which its activation stimulates an increase in GIPR expression. The physiological significance of this is obscure, since at low glucose levels GIP is not effective in stimulating insulin secretion; in the presence of 0 mM glucose, however, GIP is able to stimulate adenylyl cyclase and activate MAP kinase and PLA₂. Thus, it is probable 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; it follows that fat should also be able to regulate GIPR expression. In this manner, stimulation of PPAR α by free fatty acids derived from the adipocyte during the interdigestive period or early in the prandial process may prepare the β cell for the ensuing GIP stimulation.

Recent data from our laboratory show that GIP stimulates fatty acid oxidation within the pancreatic β cell; thus, GIP may act to prime the β cell with ATP by using intracellular fat stores. This would allow a more rapid glucose stimulation of insulin secretion postprandially.

When glucose levels are high, we see a dramatic and reproducible down-regulation of the GIPR *in vivo* and *in vitro*. However, fat is no longer able to elevate GIP receptor expression at high glucose levels. This is physiologically reasonable if GIP is acting to cause fat oxidation within the β cell. 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; as a consequence, expression of the GIP receptor is down-regulated and GIP becomes ineffective. The down-regulation occurs extremely quickly, with a discernable difference seen after only 2 h in high glucose. Our group has shown that GIP receptors are quickly internalized in response to GIP, with a significant reduction in cell surface receptors occurring after only 10 min of exposure to GIP. Thus, the “off switch” for GIP receptor activity within minutes of activation is phosphorylation; within hours, GIP receptor activity is controlled by the level of cell surface expression. Therefore, in the case of the GIPR, desensitization and down-regulation are not mutually exclusive.

Finally, in type 2 diabetes, the marked reduction in the insulinotropic potency of GIP is primarily due to down-regulation of the GIPR on the β cell as a direct result of the hyperglycemia that occurs in this condition. FJ