Differentiation Between Glucose-Induced Desensitization of Insulin Secretion and β-Cell Exhaustion in the HIT-T15 Cell Line

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Refractoriness of the pancreatic β-cell to glucose stimulation plays a role in the secretory defect of NIDDM, but the mechanisms underlying this refractoriness are not clear. The purpose of this study was to determine whether the HIT-T15 pancreatic β-cell line can be used as an investigative model for refractoriness of glucose-induced insulin secretion and, if so, whether the mechanism for this refractoriness involves alteration in stimulus-secretion coupling (desensitization) or results from exhaustion of insulin stores. In perifusion experiments, acute insulin responses (AIRs) in HIT-T15 cells progressively diminished during consecutive 5-min glucose (11.1 mmol/l) pulses (G) given every 20 min (G_1 = 9.2 ± 1.3, G_2 = 4.1 ± 1.0, G_3 = 2.7 ± 0.7, G_4 = 2.5 ± 1.1 μU/ml). To determine whether this refractoriness for glucose extended to the potentiating effects of glucose on nonglucose secretagogues, cells were challenged with arginine after desensitization with glucose. In HIT-T15 cells, the response to the arginine pulse (16.7 ± 5.2 μU/ml) after three glucose pulses was significantly less (P < 0.01) than the response to a control arginine pulse (29.6 ± 12.1 μU/ml) preceded by an infusion of buffer in the absence of glucose pulses. Variable rest periods after desensitization allowed recovery of the AIR in HIT-T15 cells; responses 30, 60, 90, and 120 min after the desensitization procedure increased in a stepwise fashion (3.8 ± 2.7, 4.5 ± 2.7, 7.8 ± 5.2, and 9.7 ± 5.3 μU/ml, respectively). To differentiate desensitization from exhaustion of insulin stores, studies were performed in the presence of epinephrine, a potent inhibitor of insulin secretion. In HIT-T15 cells, after three pulses of glucose during the epinephrine infusion, epinephrine was discontinued and the insulin response to a fourth pulse was assessed. The G_4 AIR (1.9 ± 0.6 μU/ml) was markedly less than a control G_4 AIR (5.4 ± 1.2 μU/ml) that followed an epinephrine infusion alone with no concurrent glucose pulses. β-cell refractoriness was also induced in the HIT-T15 cell using 45-min steady-state infusions of glucose. Cells were exposed to a 45-min infusion of either 3.7 or 11.1 mmol/l glucose, rested for 20 min in the absence of glucose, and then challenged with a 5-min, 11.1 mmol/l glucose pulse. In both cases, the AIR to the 5-min pulse (10.2 ± 5.1 and 2.9 ± 1.4 μU/ml after the 3.7 and 11.1 mmol/l infusion, respectively) was lower than the AIR to a control pulse (27.4 ± 5.9 μU/ml) not preceded by glucose infusion. These studies demonstrated that the HIT-T15 cell line is an appropriate model for studying mechanisms of β-cell refractoriness to glucose signaling. The short-term intensive glucose stimulation paradigms used in our studies induced an abnormality in insulin secretion that is consistent with desensitization but not β-cell exhaustion. *Diabetes* 47:606–611, 1998

The first phase of glucose-induced insulin secretion, or acute insulin response (AIR), is absent in NIDDM patients who have fasting hyperglycemia (1). This impairment is specific for glucose, as first-phase insulin secretion in response to other secretagogues remains intact (2). Furthermore, this impairment may be secondary to hyperglycemia itself because it is partially reversible after restoration of normoglycemia (3–6). This hypothesis is consistent with both in vivo and in vitro experiments demonstrating that normal pancreatic β-cells subjected to either repeated brief (7–13) or prolonged (14–16) exposure to high glucose concentrations develop diminished insulin responses to subsequent glucose challenges. Although this general phenomenon has been variably termed glucose toxicity, glucose desensitization, or β-cell exhaustion, we have argued for not using these terms interchangeably (16). We use the term "glucose toxicity" to imply nonphysiological and potentially irreversible cellular damage caused by chronic exposure to high glucose concentrations. Results from studies in our laboratory have suggested that one mechanism of glucose toxicity occurs at the level of insulin gene transcription and involves the loss of two important transcription factors, PDX-1(STF-1) and RIPE-3b1 activator (17–22). We use the terms "glucose desensitization" and "β-cell exhaustion," on the other hand, to imply a more temporary physiological state of cellular refractoriness induced by repeated or prolonged exposure to high glucose concentrations that is reversed upon the restoration of euglycemia. For the purposes of this study, we define these latter two terms in this manner: glucose desensitization refers to an alteration in stimulus-secretion coupling, whereas β-cell exhaustion refers to depletion of insulin content or stores.

Because of the ease with which large numbers of these cells can be grown and maintained in culture, identification of a cell line that appropriately models cellular refractoriness

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AIR, acute insulin response; K^+_{ATP} channel, ATP-sensitive potassium channel; IBMX, 3-isobutyl-1-methylxanthine; KRBB, Krebs-Ringer bicarbonate buffer.

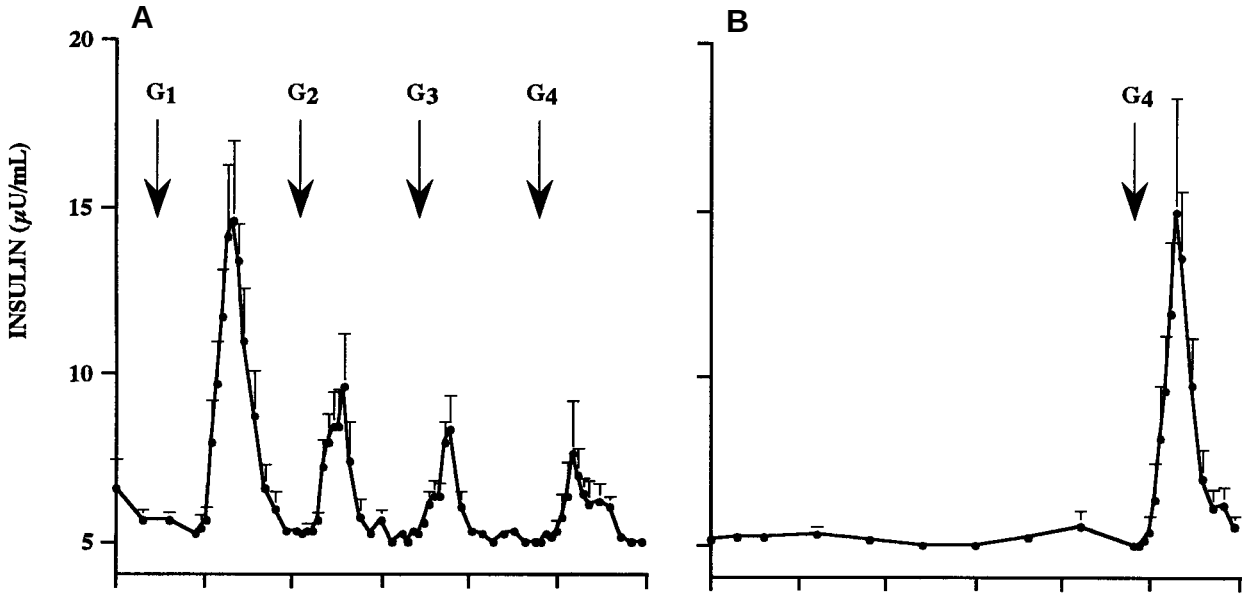

FIG. 1. Insulin responses of HIT-T15 cells to consecutive glucose pulses in perifusion experiments. Arrows indicate the start of a 5-min pulse of 11.1 mmol/l glucose and 0.1 mmol/l IBMX. Perifusion of the HIT-T15 cell (n = 5): AIRs progressively and significantly (P < 0.02) diminished during four consecutive glucose pulses (graph A); the AIR to a control G_4 given after a 70-min incubation in the absence of glucose did not differ from G_1 (graph B).

to glucose signaling should facilitate the study of these phenomena. Differentiation of glucose desensitization and β-cell exhaustion has not been examined in any of the available glucose-responsive, insulin-secreting β-cell lines. Therefore, the purpose of our study was to determine whether refractoriness of glucose-induced insulin secretion can be induced in the HIT-T15 pancreatic β-cell line in response to consecutive brief pulses or steady-state infusions of glucose, and whether this phenomenon is caused by desensitization or exhaustion of β-cell insulin stores.

RESEARCH DESIGN AND METHODS

Cell culture. The HIT-T15 cell line, originally developed by Santerre et al. (23), was provided by A.E. Boyd III (Baylor University, Houston, TX). Experiments were performed on passages 73–82 because these passages have intact glucose-responsive insulin secretion (17). The cells were maintained in RPMI-1640 supplemented with 10% fetal calf serum and 11.1 mmol/l glucose. They were grown in a humidified

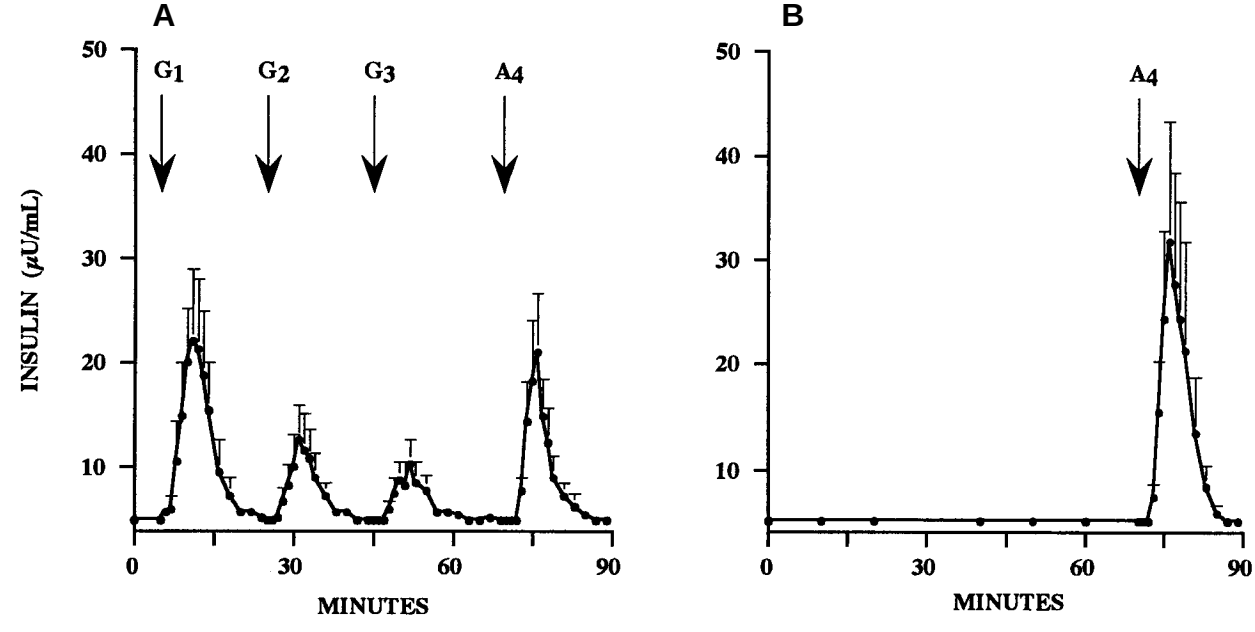

FIG. 2. Insulin responses of HIT-T15 cells to an arginine challenge after desensitization. Arrows indicate the start of 5-min pulses. After three pulses of 11.1 mmol/l glucose and 0.1 mmol/l IBMX (G_1–G_3), cells were challenged with one pulse of 20 mmol/l arginine, 0.8 mmol/l glucose, and 0.1 mmol/l IBMX (A_4). The response to arginine (graph A) was not significantly different from the response to control arginine challenge (graph B), which was not preceded by glucose pulses.

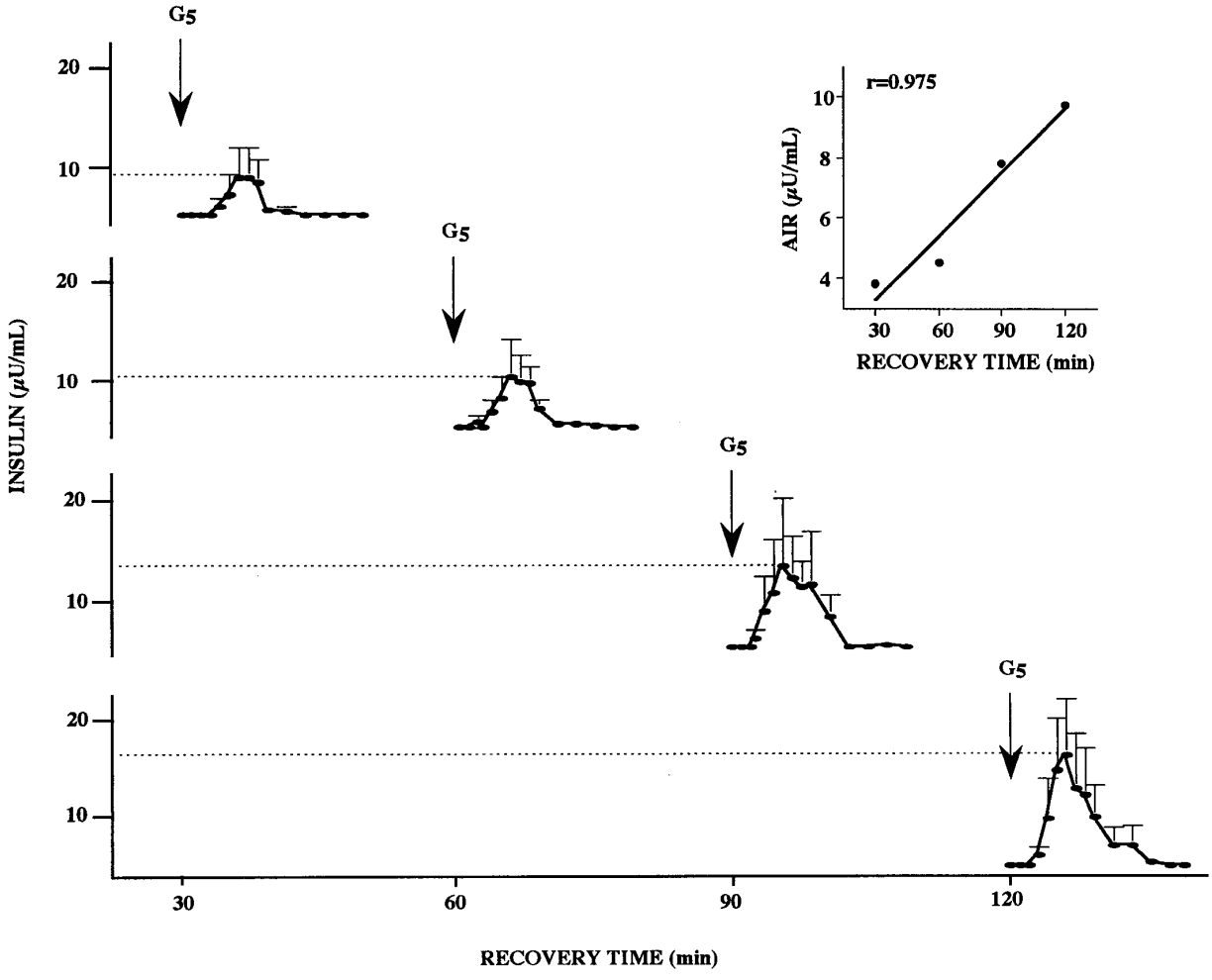

FIG. 3. Time course of the recovery of insulin secretion in the HIT cell after desensitization. Following the four-pulse protocol, cells were perifused in the absence of glucose for variable time periods and subsequently rechallenged with a fifth glucose pulse. Arrows indicate the start of a 5-min pulse of 11.1 mmol/l glucose and 0.1 mmol/l IBMX. Recovery time refers to the amount of time elapsed since G_4. AIRs at 30, 60, 90, and 120 min after desensitization increased in a stepwise fashion. The inset shows that the recovery of insulin secretion was directly correlated with time (r = 0.975, P < 0.05).

atmosphere of 5% CO_2/95% air at 37°C. The medium was changed every 48–72 h. The cells were passaged weekly after detachment using trypsin-EDTA. Before each experiment, cells were plated on sterile 18-mm round coverslips at a cell density of approximately 1.0–1.5 × 10^6 and maintained in 12-well culture dishes for 2–3 days.

Perifusion experiments. A perifusion apparatus was built according to the description of Hill and Boyd (24). Coverslips with cells attached were placed in 25-mm Swinnex chambers (Millipore, Bedford, MA) filled with Krebs-Ringer bicarbonate buffer (KRBB) containing 118.5 mmol/l NaCl, 2.45 mmol/l $CaCl_2 \cdot 2H_2O$, 1.19 mmol/l KH_2PO_4, 4.74 mmol/l KCl, 25 mmol/l $NaHCO_3$, 1.19 mmol/l $MgSO_4 \cdot 7H_2O$, 10 mmol/l HEPES, and 0.1% bovine serum albumin (pH 7.4). The chambers were then placed in a 37°C water bath. Buffer was pumped from beakers maintained in the water bath to the chambers through tubing connected to a Gilson Minipuls 2 pump (Middleton, WI) at a flow rate of 1 ml/min. Effluent fractions were collected for insulin assay. At the start of each experiment, cells were rested in the absence of glucose for 30 min with KRBB. Two types of glucose stimulation were used: glucose pulses (G) and continuous glucose infusion. Each glucose pulse lasted 5 min and was followed by a 15-min rest period. Glucose infusions lasted 45 min and were followed by a 20-min rest period. The glucose buffer consisted of KRBB containing 11.1 mmol/l glucose and 0.1 mmol/l 3-isobutyl-1-methylxanthine (IBMX), except where noted. This concentration has been shown to be stimulatory for the HIT-T15 cell line (25). The arginine pulses (A) also lasted 5 min and consisted of KRBB containing 20 mmol/l arginine, 0.8 mmol/l glucose, and 0.1 mmol/l IBMX. Inhibition of glucose-induced insulin secretion was achieved using KRBB containing 10^{-6} mol/l epinephrine, a concentration that has been shown to be inhibitory for the HIT-T15 (26) cell line. Cells were exposed to epinephrine for 5 min before and during stimulation, and the epinephrine was removed 20 min before final glucose stimulation. During each pulse/rest period, 1-min fractions were collected for the first 10 min and 2-min fractions were collected for each subsequent 10-min period. When cells were not stimulated for periods in excess of 1 h, samples were collected every 10 min.

Assays. Insulin concentrations in the samples were determined by radioimmunoassay, as previously described (27). Epinephrine levels were determined using the single-isotope radioenzymatic assay, as described by Evans et al. (28).

Expression of data and statistics. Data are means ± SE. The AIR was calculated as the mean of the three peak values minus the basal value. The basal value was determined immediately before the first stimulation period. Data were analyzed by analysis of variance or Student's paired t test where appropriate. $P < 0.05$ was considered significant.

RESULTS

To determine whether the HIT-T15 cell becomes refractory to glucose signaling with repeated brief exposures to high glucose concentrations, insulin responses to four consecutive glucose pulses were assessed in perifusion experiments (Fig. 1*A*). The AIRs progressively and significantly (P < 0.0001) diminished during the four pulses: G_1 = 9.2 ± 1.3, G_2 = 4.1 ± 1.0, G_3 = 2.7 ± 0.7, and G_4 = 2.5 ± 1.1 μU/ml (n = 5 sets of four pulses). We determined that this diminution was not an artifact of the cells' prolonged exposure to perifusion because the AIR to a control G_4 glucose challenge, given after a 70-min

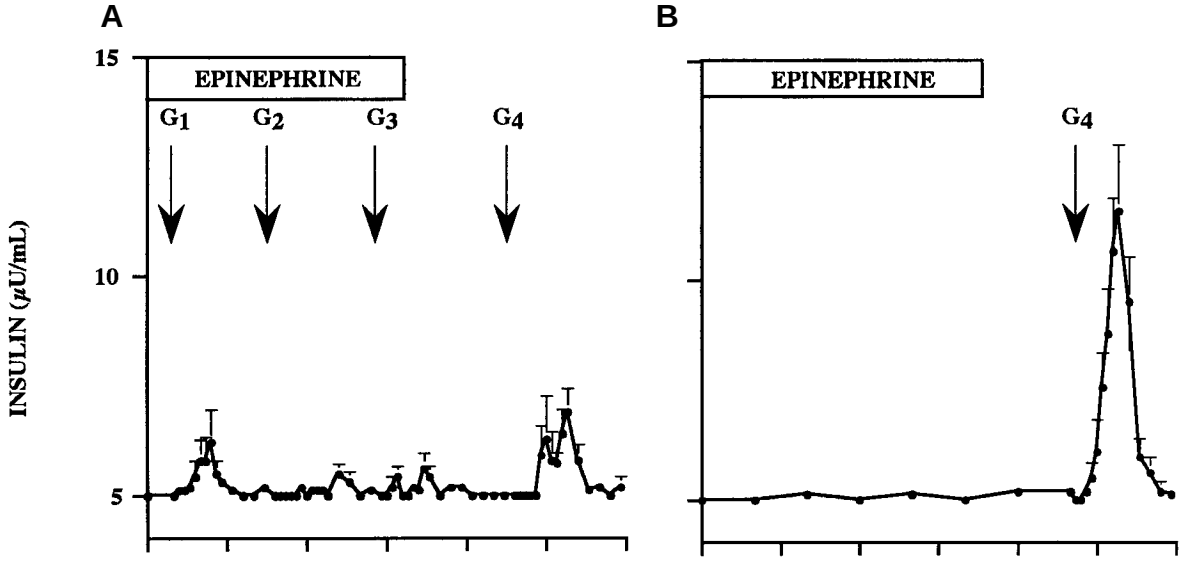

FIG. 4. Insulin responses of HIT-T15 cells to consecutive glucose pulses in the presence of epinephrine (n = 5). The epinephrine infusion concentration was 10^{-6} mol/l. Arrows indicate the start of a 5-min pulse of 11.1 mmol/l glucose and 0.1 mmol/l IBMX. Despite nearly complete inhibition of the insulin response to G_1–G_3 by epinephrine (graph *A*), the response to G_4 was less than the response to the control G_4 (graph *B*), which was preceded by the epinephrine infusion without concurrent glucose pulses.

incubation in the absence of glucose pulses (Fig. 1*B*), was not significantly different from the G_1 response: 8.6 ± 2.5 vs. 9.2 ± 1.3 μU/ml, respectively (NS; n = 5).

We next addressed whether this refractoriness for glucose extended to the ability of glucose to potentiate arginine-induced insulin secretion. HIT-T15 cells were exposed to three consecutive glucose pulses and then challenged with a pulse of arginine in a buffer containing 0.8 mmol/l glucose (Fig. 2*A*). The response to A_4 (16.7 ± 5.2) was significantly less (62 ± 8%; n = 4; P < 0.01) than the response to a control A_4 (29.6 ± 12.1) (Fig. 2*B*), which was preceded by an infusion of buffer in the absence of glucose pulses.

To determine whether this refractoriness was reversible, HIT-T15 cells were incubated in the absence of glucose for variable time periods after the four-pulse desensitization protocol and then challenged with a fifth glucose pulse (Fig. 3). Responses to G_5 at 30, 60, 90, and 120 min after desensitization increased in a stepwise fashion (3.8 ± 2.7, 4.5 ± 2.7, 7.8 ± 5.2, and 9.7 ± 5.3 μU/ml, respectively). Recovery of the AIR was directly correlated with time (Fig. 3, inset; n = 3; r = 0.975; P < 0.05).

To differentiate desensitization from exhaustion, the desensitization protocol was performed in the presence of epinephrine, given to inhibit insulin secretion. First an infusion of epinephrine was given during G_1–G_3; then epinephrine was discontinued and the AIR to a fourth glucose pulse was determined (Fig. 4*A*). As expected, the epinephrine infusion nearly completely inhibited the insulin response to G_1–G_3; however, even after discontinuation of the epinephrine infusion, the response to G_4 (1.9 ± 0.6 μU/ml) was significantly less (P < 0.05) than the control G_4 AIR (5.4 ± 1.2 μU/ml; n = 5) that followed an epinephrine infusion without concurrent glucose pulses (Fig. 4*B*).

Because the control G_4 AIR after the epinephrine infusion was lower than the G_4 AIR after incubation in buffer without epinephrine, epinephrine concentrations in effluent fractions from the perifusion apparatus were determined in the HIT-T15 cell experiments. Samples were taken before and during the epinephrine infusion and during the G_4 pulses. As is shown in Fig. 5, there was residual epinephrine in the effluent fractions during the G_4 pulses, which would explain the dampened insulin response to G_4.

We next addressed whether glucose refractoriness could be induced in the HIT-T15 cell in a similar time frame using steady-state glucose infusions rather than consecutive brief pulses. HIT-T15 cells were exposed to a 45-min infusion of either 3.7 or 11.1 mmol/l glucose, rested for 20 min in the absence of glucose, and then challenged with a 5-min 11.1-mmol/l glucose pulse. The 3.7-mmol/l glucose concentration

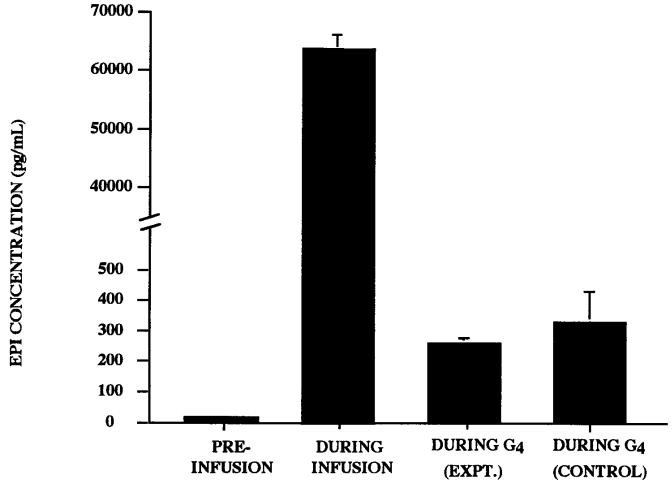

FIG. 5. Epinephrine concentrations in effluent fractions before and during epinephrine infusions and during G_4 pulses in the HIT-T15 cell experiments. Residual epinephrine was seen in the fractions collected during the G_4 pulses.

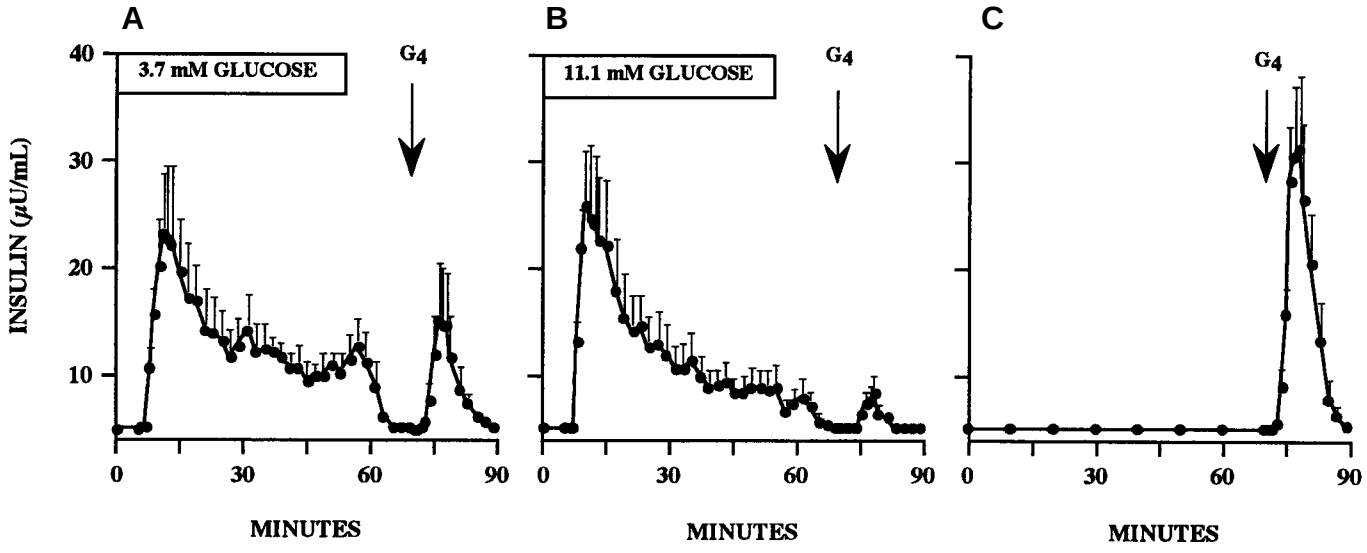

FIG. 6. Insulin responses of HIT-T15 cells to steady-state infusions of glucose and a subsequent 5-min glucose pulse. Cells were exposed to 45-min infusions of either 3.7 mmol/l glucose plus 0.03 mmol/l IBMX (graph *A*) or 11.1 mmol/l glucose plus 0.1 mmol/l IBMX (graph *B*). Control cells (graph *C*) were perifused with buffer containing no glucose. Arrows indicate the start of a 5-min pulse of 11.1 mmol/l glucose and 0.1 mmol/l IBMX. The amount of insulin secreted in response to the two infusions was indistinguishable, yet the cells exposed to the higher infusion rate had a smaller AIR to G_4.

was chosen because this infusion contained the sum of the glucose given during G_1–G_3 in the pulse desensitization protocol, and the 11.1 mmol/l glucose concentration was chosen because it was the magnitude of the pulses. In both cases, the AIR to the 5-min pulse (10.2 ± 5.1 and 2.9 ± 1.4 μU/ml after the 3.7 and 11.1 mmol/l infusion, respectively; $n = 4$) (Fig. 6*A* and *B*, respectively) was significantly lower ($P < 0.05$) than the AIR to the control pulse (27.4 ± 5.9 μU/ml; $n = 4$) (Fig. 6*C*), which followed incubation in buffer containing no glucose. Interestingly, the amount of insulin secreted in response to the two infusions was indistinguishable, yet the AIRs to the subsequent glucose pulse differed, with the cells exposed to the higher infusion rate having a smaller response to G_4.

DISCUSSION

This study demonstrated that glucose refractoriness can be induced in the HIT-T15 pancreatic β-cell line in perifusion experiments using repetitive brief pulses of glucose. This state of β-cell refractoriness meets the criteria for desensitization, since it reverses in a linear, time-dependent manner within 120 min of culturing the cells in the absence of glucose. The refractoriness for glucose extends to its potentiating effects on a nonglucose secretagogue because, after the repeated pulses of glucose, the insulin response to arginine in a buffer containing glucose was significantly decreased. Thus the glucose-desensitized cells lost at least a part of their ability to potentiate the arginine response (29–31), but the glucose-independent portion of the arginine response remained intact. It is important that this refractoriness was not prevented when we blocked insulin secretion with epinephrine, which then allowed us to differentiate between the desensitizing effect of a repeated glucose signal and depletion of insulin stores (β-cell exhaustion) as a consequence of repeated insulin secretion. The slightly attenuated insulin response to the control G_4 after the epinephrine infusion was stopped is explained by the residual epinephrine in the effluent fractions during the G_4 glucose challenge. This state of refractoriness was also induced in perifusion experiments using longer steady-state infusions of glucose. The glucose infusion experiments provided an additional line of evidence supporting the concept that glucose desensitization is caused by an alteration in stimulus-secretion coupling rather than exhaustion. The amount of insulin secreted in response to the 11.1 and 3.7 mmol/l glucose infusions was indistinguishable, yet the AIRs to G_4 differed between the two groups. The cells exposed to the higher glucose concentration had a lower AIR to G_4 and thus were desensitized to a greater extent than the cells exposed to the lower glucose concentration. These data lead to our conclusion that repeated stimulation with glucose in this cell line causes β-cell refractoriness that is explicable by desensitization rather than depletion of insulin stores, and further suggest that glucose desensitization occurs in a dosage-dependent fashion.

The HIT-T15 cell has been widely used in the study of insulin secretion for over a decade. Early studies with this cell line established that it secretes insulin in response to glucose and other β-cell secretagogues (23–25). Studies in our laboratory have shown that the HIT-T15 cell loses the ability to secrete insulin after chronic culture in high glucose concentrations, an event we refer to as glucose toxicity. Our present study uniquely demonstrates that this cell line also appropriately models glucose desensitization. Having a model that can be maintained with ease in culture should facilitate the study of this secretory phenomenon.

The insulin secretion pattern of the HIT-T15 cell seen with our in vitro glucose pulse desensitization protocol is similar to the pattern observed in vivo in human subjects given two consecutive intravenous glucose pulses 20–30 min apart (7–9). In these studies, the response to the second glucose pulse was markedly less than the response to the first. Refractoriness of the β-cell to consecutive glucose pulses

has also been demonstrated in vitro in perfused rat pancreases (11–13) and perifused rat islets (10).

Efendic et al. (9) used an approach similar to our experiments with epinephrine to determine if the diminished insulin response seen in humans after consecutive glucose pulses was caused by defective glucose signaling or defective release of insulin. They reported that co-infusion of somatostatin with the first glucose pulse blocked the insulin response to the first load, but did not prevent diminution of the response to the second pulse. They concluded that it was the repeated glucose signal, and not the repeated insulin release, that caused the diminution. More recently, however, Sako and colleagues (32,33), and Leahy et al. (31) came to a different conclusion from experiments using diazoxide, which blocks insulin secretion by maintaining open ATP-sensitive potassium channels (K^+_{ATP} channels). Both groups found that infusion of diazoxide during prolonged hyperglycemic conditions prevented the β-cell from becoming desensitized to glucose, and concluded that the refractoriness they were studying may have been caused by excessive insulin secretion. One possible explanation for this discrepancy is that the site of desensitization in the β-cell may occur at or distal to the K^+_{ATP} channel. By preventing the closure of the K^+_{ATP} channel, diazoxide also prevents all steps in the glucose-induced insulin secretion pathway distal to this channel (i.e., depolarization of the cell, opening of Ca^{2+} channels, Ca^{2+} influx, and exocytosis of insulin vesicles). Therefore, in light of the studies using diazoxide, results from the studies we described herein suggest that β-cell desensitization is not the result of an alteration in the pathway proximal to the K^+_{ATP} channel (i.e., glucose metabolism) and point to the possibility that this desensitization may occur at a later step in the stimulus-secretion pathway.

In summary, the present study demonstrated that the HIT-T15 cell line is an appropriate model for studying glucose desensitization. The experimental paradigms we used induced a refractoriness to glucose signals that we interpreted to be consistent with glucose desensitization but not β-cell exhaustion. This cell line should serve as a convenient model in future investigations of the precise mechanism(s) of this secretory impairment and may help clarify its role in the pathogenesis of NIDDM.

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