Isolation of INS-1–Derived Cell Lines With Robust ATP-Sensitive K^+ Channel-Dependent and -Independent Glucose-Stimulated Insulin Secretion

Hans E. Hohmeier, Hindrik Mulder, Guoxun Chen, Rosemarie Henkel-Rieger, Marc Prentki, and Christopher B. Newgard

The biochemical mechanisms involved in regulation of insulin secretion are not completely understood. The rat INS-1 cell line has been used to gain insight in this area because it secretes insulin in response to glucose concentrations in the physiological range. However, the magnitude of the response is far less than that seen in freshly isolated rat islets. In the current study, we have stably transfected INS-1 cells with a plasmid containing the human proinsulin gene. After antibiotic selection and clonal expansion, 67% of the resultant clones were found to be poorly responsive to glucose in terms of insulin secretion (≤2-fold stimulation by 15 mmol/l compared with 3 mmol/l glucose), 17% of the clones were moderately responsive (2- to 5-fold stimulation), and 16% were strongly responsive (5- to 13-fold stimulation). The differences in responsiveness could not be ascribed to differences in insulin content. Detailed analysis of one of the strongly responsive lines (832/13) revealed that its potent response to glucose (average of 10-fold) was stable over 66 population doublings (~7.5 months of tissue culture) with half-maximal stimulation at 6 mmol/l glucose. Furthermore, in the presence of 15 mmol/l glucose, insulin secretion was potentiated significantly by 100 μmol/l isobutylmethylxanthine (320%), 1 mmol/l oleate/palmitate (77%), and 50 nmol/l glucagon-like peptide 1 (60%), whereas carbachol had no effect. Glucose-stimulated insulin secretion was also potentiated by the sulfonylurea tolbutamide (threefold at 3 mmol/l glucose and 50% at 15 mmol/l glucose) and was abolished by diazoxide, which demonstrates the operation of the ATP-sensitive K^+ channel (K_{ATP}) in 832/13 cells. Moreover, when the K_{ATP} channel was bypassed by incubation of cells in depolarizing K^+ (35 mmol/l), insulin secretion was more effectively stimulated by glucose in 832/13 cells than in parental INS-1 cells, which demonstrates the presence of a K_{ATP} channel-independent pathway of glucose sensing. We conclude that clonal selection of INS-1 cells allows isolation of cell lines that exhibit markedly enhanced and stable responsiveness to glucose and several of its known potentiators. These lines may be attractive new vehicles for studies of β-cell function. *Diabetes* 49:424–430, 2000

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Received for publication 27 August 1999 and accepted in revised form 10 November 1999.

GLP-1, glucagon-like peptide 1; HBSS, HEPES balanced salt solution; HPLC, high-performance liquid chromatography; IBMX, isobutylmethylxanthine; K_{ATP}, ATP-sensitive K^+ channel; PD, population doubling; RIA, radioimmunoassay.

The biochemical mechanisms involved in fuel-stimulated insulin secretion are not completely understood (1–3). One impediment to gaining full understanding in this area has been the procurement of insulinoma cell lines that faithfully and stably mimic the performance of β-cells within the normal pancreatic islets of Langerhans. Numerous rodent β-cell lines exhibiting different degrees of differentiation have been reported (3,4). These range from the poorly differentiated RINm5F cell line, which has a low insulin content and no glucose-stimulated insulin secretion (5), to rat cell lines such as INS-1 and mouse cell lines such as MIN-6, βTC6-F7, and βHC9, which have an insulin content closer to that of normal islets and retain some glucose-stimulated insulin secretion (6–10). However, even the best rodent cell lines are imperfect. For example, INS-1 cells generally exhibit only a 2- to 4-fold increase in insulin secretion in response to glucose (6,11,12), which is far less than the 15-fold responses achievable with freshly isolated primary islets (13). Also, MIN-6 cells exhibit secretory responses to pyruvate, which is not a secretagogue for normal islets (14), and βHC9 cells grow very slowly and are thus difficult to study. Finally, loss of differentiated features as a function of time in tissue culture has been reported for several rodent cell lines, including RIN1046-38 and βTC6 (8,15,16). Genetic engineering of RIN1046-38 cells results in clones with stable glucose responsiveness but with maximal insulin secretion occurring at subphysiological glucose concentrations because of a high level of low K_m hexokinase activity in these cells (16–18). Stable glucose responsiveness has also been reported for βTC cells after clonal selection in soft agar (8), but even these cloned cell lines (e.g., βTC6-F7) appear to lose glucose responsiveness after prolonged tissue culture (19).

We have worked extensively with INS-1 cells as a model for metabolic signaling mechanisms in the β-cell (11,12,20,21) and have found that fresh aliquots of these cells at relatively low

passage numbers exhibit two- to fourfold responses to glucose over the physiological range, as reported in the study by Asfari et al. (6) that described their isolation. However, further growth of these cells in culture for 2–3 months results in a reduction of the glucose response to twofold or less. INS-1 cells were originally isolated by dispersion of a transplantable radiation-induced INS tumor from NEDH rats into a tissue culture medium containing β-mercaptoethanol (6). Based on the description of methods used in this process (6), INS-1 cells may not be clonal and may instead represent a mixture of endocrine cells with distinct phenotypes. If this is correct, then the loss of differentiated function that we have experienced with INS-1 cells may be explained by preferential expansion of a glucose-unresponsive subpopulation of cells from an initial polyclonal mixture.

To test this possibility, we used a stable transfection strategy to isolate a large number of discrete INS-1–derived colonies. Analysis of the resultant clones shows that the original INS-1 cell line is clonally heterogeneous but also that cell lines with robust secretory responses to glucose can be derived from stable transfection of the original population. These new lines may serve as improved models for studies of β-cell function.

RESEARCH DESIGN AND METHODS

Reagents. All reagents were from Sigma (St. Louis, MO) unless otherwise noted and were used at the concentrations shown in the legends to the figures.

Cell culture. Parental INS-1 cells (6) were a generous gift from Dr. Claes Wollheim (Geneva). These cells and new cell lines derived from them by stable transfection were grown in 10-cm tissue culture dishes at 37°C and 5% CO_2 in a humidified atmosphere. The cells were passaged every 5 days by using 1 ml 0.05% trypsin-EDTA. The culture medium was RPMI-1640 with 11.1 mmol/l D-glucose supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 10 mmol/l HEPES, 2 mmol/l L-glutamine, 1 mmol/l sodium pyruvate, and 50 µmol/l β-mercaptoethanol.

Isolation of new INS-1–derived cell lines with a stable transfection approach. To investigate clonal heterogeneity in INS-1 cells while increasing insulin content, the parental cells were stably transfected with the plasmid pCMV8/INS/IRES/Neo containing the human insulin cDNA as previously described (17). Two independent transfection experiments were performed, each yielding 50–60 discrete colonies that appeared under selection with G418. These colonies were isolated and expanded for further analysis.

Secretion assays. Parental INS-1 cells or cell lines derived from them by using stable transfection were used in these studies. In studies involving the new cell line 832/13, population doublings (PDs) 26–92 were used. The cells were plated onto 24-well plates at a density of ~0.5×10^6 cells/well and were grown to 100% confluence before assay. At 18 h before secretion experiments, the standard tissue culture medium containing 11.1 mmol/l glucose was switched to fresh medium containing 5 mmol/l glucose. Insulin secretion was assayed in HEPES balanced salt solution (HBSS) (114 mmol/l NaCl, 4.7 mmol/l KCl, 1.2 mmol/l KH_2PO_4, 1.16 mmol/l $MgSO_4$, 20 mmol/l HEPES, 2.5 mmol/l $CaCl_2$, 25.5 mmol/l $NaHCO_3$, and 0.2% bovine serum albumin [essentially fatty acid free], pH 7.2). Cells were washed in 1 ml HBSS with 3 mmol/l glucose followed by a 2-h preincubation in 2 ml of the same buffer. Insulin secretion was then measured by using static incubation for a 2-h period in 0.8 ml of HBSS containing the glucose concentrations and/or secretagogues indicated in the figure legends. For studies of K_{ATP} channel-independent insulin secretion (22,23), assays were performed as described above except that 35 mmol/l KCl (depolarizing K^+) was included; consequently, the Na^+ concentration was reduced from 120 to 89.8 mmol/l to maintain osmolarity.

Insulin radioimmunoassay. For determination of total insulin content and for measurement of absolute output of insulin during glucose stimulation of INS-1 lines, samples were analyzed by radioimmunoassay (RIA) with the ImmunoChem Coated Tube Insulin RIA kit (ICN Pharmaceuticals, Costa Mesa, CA). According to the manufacturer, this assay detects both human and rat insulins with a relative reactivity toward rat insulin of 90% compared with human insulin. In all other secretion experiments in which data are expressed as fold increases, the Coat-a-Count kit (Diagnostic Products, Los Angeles, CA) was used as previously described (17,18). This assay uses antibodies to human insulin that cross-react ~20% with rat insulin. Human insulin (referenced to U.S. Pharmacopia, Rockville, MD, insulin lot G) was used for standard curves in both assays.

Insulin content and proinsulin processing. To measure cellular insulin content, cell pellets were sonicated in 1 mol/l acetic acid containing 0.1% bovine serum albumin. Aliquots of acid extracts were subjected to RIA with an assay that detects both rat and human insulin (ICN kit) as described above. The extent of proinsulin processing was evaluated by preparing acetic acid extracts of whole cells or culture medium and by analyzing these samples with high-performance liquid chromatography (HPLC) as previously described (17,24).

RESULTS

Clonal heterogeneity of INS-1 cells revealed by stable transfection with the human insulin gene and isolation of individual clones. Parental INS-1 cells were transfected with a plasmid containing the human insulin gene under control of the cytomegalovirus promoter and a neomycin resistance gene. A total of 58 independent colonies were isolated after selection with G418. Colonies in G418-containing medium were discrete and well separated and likely represented populations derived from individual clones. All 58 cell lines were screened by measuring the fold increase in insulin secretion at 15 vs. 3 mmol/l glucose. As shown in Fig. 1, 67% of the clones (39 clones) were poorly responsive to glucose (≤2-fold stimulation by high glucose), 17% of the clones (10 clones) were moderately responsive (2- to 5-fold stimulation), and 16% (9 clones) were strongly responsive (5- to 13-fold stimulation). These results are representative of two independent transfection experiments and indicate that parental INS-1 cells consist of a mixture of cells with different glucose-sensing capacity.

To validate the results obtained in the screen shown in Fig. 1, glucose-stimulated insulin secretion was reevaluated in four "poorly responsive" clones (lines 832/1, 832/2, 832/7, and 832/23) and in four "strongly responsive" clones (lines 832/3, 832/13, 832/21, and 832/24). Insulin secretion was measured with an RIA that detects rat and human insulin equally. The data in Fig. 2 confirm the distinction between the two groups of clones in that the poorly responsive lines were similar to or less responsive than the parental cells, which exhibited a threefold increase in insulin secretion as glucose was increased from 3 to 15 mmol/l. In contrast, all four of the strongly responsive clones exhibited better respon-

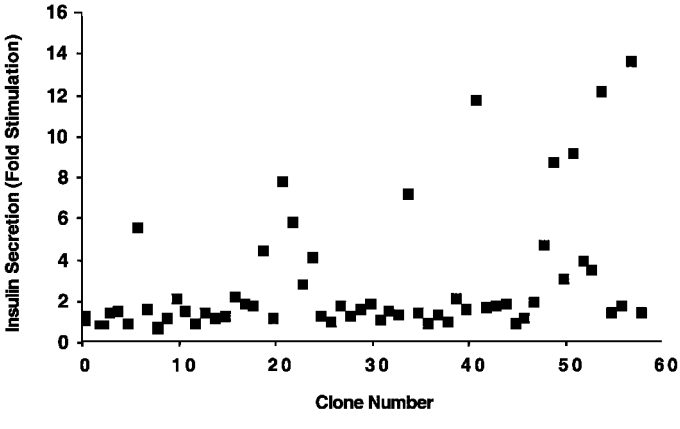

FIG. 1. Screen for glucose-responsive INS-1–derived clones. Parental INS-1 cells were stably transfected with a plasmid containing the human proinsulin gene as described in RESEARCH DESIGN AND METHODS. After antibiotic selection, individual colonies were isolated, expanded, and screened by measuring the fold increase in insulin secretion at 15 vs. 3 mmol/l glucose. Data for 58 individual clones are presented and represent the means of three independent measurements per clone.

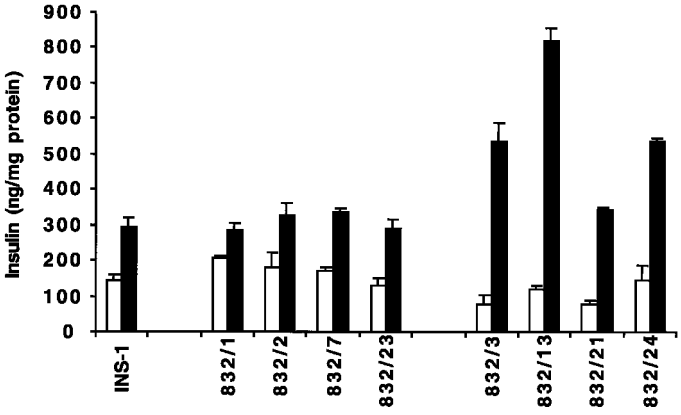

FIG. 2. Glucose-stimulated insulin secretion in INS-1–derived clones. Based on the screen shown in Fig. 1, four clones with poor glucose responsiveness (832/1, 832/2, 832/7, and 832/23) and four strongly responsive clones (832/3, 832/13, 832/21, and 832/24) were selected for further analysis. Insulin secretion was measured at 3 (□) and 15 (■) mmol/l glucose with an RIA that detects both human and rat insulins with a relative reactivity toward rat insulin of 90% compared with human insulin. Data represent the means ± SE for three independent experiments, each of which was performed in triplicate.

siveness than parental INS-1 cells (range of 4.4- to 8-fold responses to glucose). Among the highly responsive clones, line 832/13 secreted the largest amount of insulin during glucose stimulation (800 ng · mg^{-1} protein · h^{-1}) and was chosen for further study.

Insulin content of selected INS-1 cell lines. We next investigated whether the superior performance of the strongly responsive group could be related to insulin content. To evaluate this possibility, we measured total insulin content (rat + human insulin) in the clones described in Fig. 2. As shown in Table 1, insulin content was generally increased in the two groups of clones derived from stable transfection of the human insulin gene compared with parental INS-1 cells, but no consistent difference between the two groups of transfected cells was evident. In particular, the insulin content of clone 832/13, the strongly responsive line that was chosen for further study, was 1.5 µg/10^6 cells, which is similar to the content of the four poorly responsive clones (range 1.3–2.3 µg/10^6 cells).

TABLE 1
Insulin content of INS-1–derived cell lines

Response	Cell line	Insulin content (ng/10^6 cells)
2- to 4-fold	Parental INS-1	866 ± 108
Poor (≤2-fold)	832/1	1,265 ± 332
	832/2	1,322 ± 308
	832/7	2,183 ± 254
	832/23	1,722 ± 246
Strong (5- to 13-fold)	832/2	1,165 ± 281
	832/13	1,440 ± 348
	832/21	824 ± 96
	832/24	1,405 ± 111

Data are means ± SE for four independent determinations. Insulin content was measured using an RIA that detects both human and rat insulins with a relative reactivity toward rat insulin of 90% compared with human insulin. Note that, although all but one of the transfected clones (832 series) contained more insulin than the parental INS-1 cells, no consistent difference was evident in content between poorly (first group of four lines) and strongly (second group of four lines) responsive cells.

Stable glucose response and proinsulin processing in a clonal INS-1 cell line. To determine whether clone 832/13 was capable of maintaining potent glucose responsiveness in a stable fashion, insulin secretion assays were performed at 26, 64, and 92 PDs, which represent a total interval of ~7.5 months of tissue culture. As shown in Fig. 3, insulin secretion was stimulated 8-fold at 15 mmol/l glucose relative to secretion at 3 mmol/l glucose in cells at PD 26, 11-fold at PD 64, and 10-fold at PD 92. These results indicate that glucose responsiveness of the selected INS-1 cell line is stably maintained.

We also studied proinsulin processing by using HPLC analysis. As shown in Fig. 4*A*, parental INS-1 cells contained two prominent absorbance peaks. One comigrated with rat insulin I (17), and the other likely represents rat insulin II based on previous studies of INS-1 cells by Asfari et al. (6) and because insulin was measured in both peaks by RIA. Proinsulin was present at very low levels in the parental INS-1 cells, which suggests that efficient processing of the endogenous rat proinsulin occurred in our starting population (Fig. 4*A*). The 832/13 cells at PD 26 retained the rat insulin I and II peaks and also exhibited a smaller peak comigrating with a human insulin standard, which confirms expression of the stably introduced human insulin transgene (Fig. 4*B*). Again, no significant accumulation of proinsulin was evident despite overexpression of the human proinsulin gene. Importantly, human insulin expression and the efficiency of proinsulin processing were identical in 832/13 cells at PD 42 to those at PD 26 (Fig. 4*B* and *C*). This provides further evidence of a stable phenotype in cell populations selected by stable transfection.

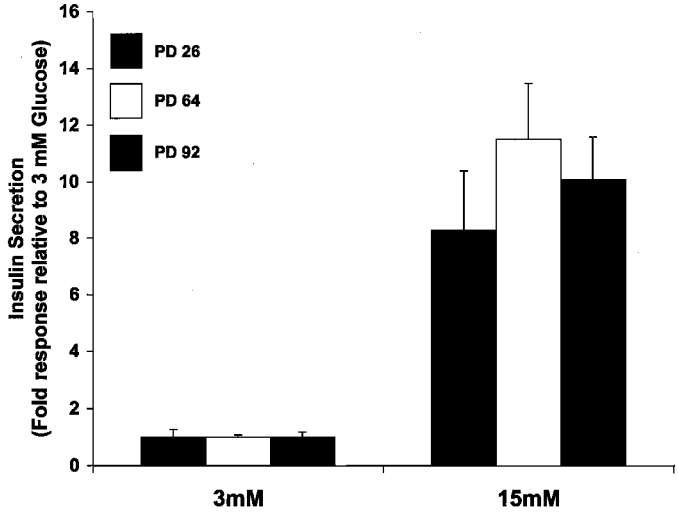

FIG. 3. Stable phenotype of INS-1–derived clone 832/13. Insulin secretion in response to 3 and 15 mmol/l glucose was measured in clone 832/13 at PD 26, PD 64, and PD 92, which indicate a total time interval of ~7.5 months of tissue culture. Insulin secreted into the medium was measured with RIA, was normalized to total cellular protein, and was expressed as fold increase relative to insulin secreted at 3 mmol/l glucose. Data represent the means ± SE of two independent experiments, each of which was performed in triplicate.

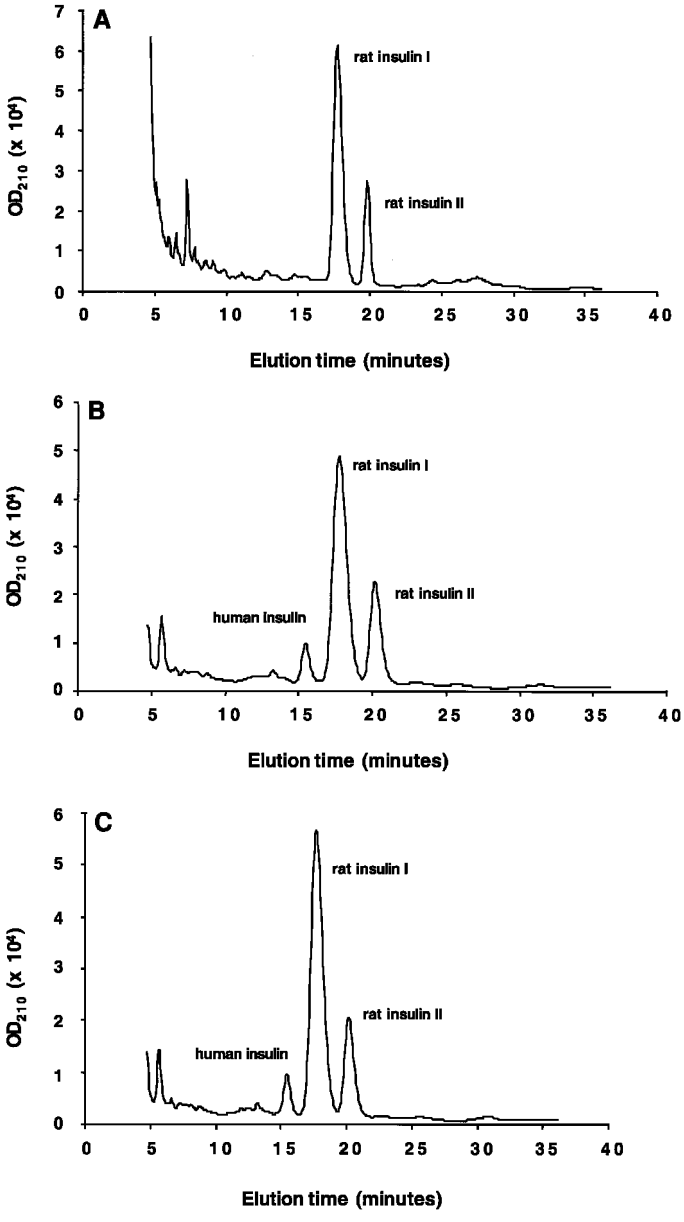

FIG. 4. HPLC analysis of proinsulin processing in INS-1–derived clones. Extracts were prepared as described in RESEARCH DESIGN AND METHODS from parental INS-1 cells (*A*), clone 832/13 at PD 26 (*B*), and clone 832/13 at PD 42 (*C*) and were subjected to HPLC analysis. Parental INS-1 cells contain two prominent absorption peaks, the larger one comigrating with rat insulin I and the other likely representing rat insulin II based on the RIA of fractions from this peak. In clone 832/13 at either PD 26 or 42, the rat insulin peaks remain, and a new peak that comigrates with human insulin appears. Importantly, these fractions contain very little proinsulin, and the HPLC profile is unaltered in 832/13 cells at PD 42 compared with PD 26, which provides further evidence of the phenotypic stability of these cells.

Glucose dose response in the 832/13 INS-1 cell line. We next evaluated insulin secretion in response to a range of glucose concentrations in 832/13 cells. As shown in Fig. 5, insulin secretion was unchanged as glucose was increased from 0 to 3 mmol/l glucose. Insulin secretion was doubled at 4 mmol/l glucose relative to secretion at 0 or 3 mmol/l and then continued to increase as the glucose concentration increased until a maximal response of 13-fold above baseline was

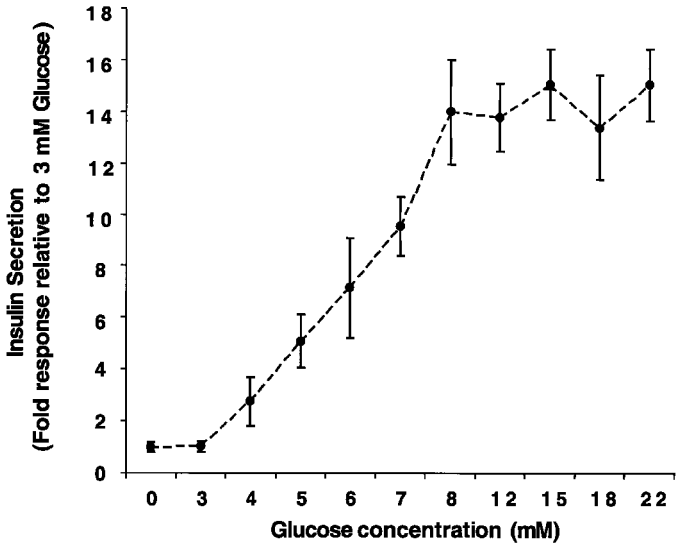

FIG. 5. Glucose dose-response curve for INS-1–derived clone 832/13. The 832/13 cells were incubated at the indicated concentrations of glucose for 2 h. Insulin secreted into the medium was measured with RIA, was normalized to total cellular protein, and was expressed as fold increase relative to insulin secreted at 3 mmol/l glucose. Data represent the means ± SE for five independent experiments.

attained at 8 mmol/l glucose. Further increases in glucose caused no additional enhancement in insulin secretion. The magnitude of the response to glucose exhibited by 832/13 cells compares favorably with the responses reported for freshly isolated rat islets (13). However, the glucose dose response was slightly shifted to the left compared with rat islets, which have a threshold for glucose response of 5.5 mmol/l and maximal responses that occur at 16–20 mmol/l glucose (2,3).

Effect of glucose potentiators on the 832/13 INS-1 cell line. Glucose regulates insulin secretion from normal pancreatic islets in concert with numerous physiologically relevant potentiators (1–3). We therefore evaluated the capacity of the 832/13 cell line to respond to such agents. Consistent with the data in Figs. 4 and 5, insulin secretion was stimulated ninefold as glucose was increased from 3 to 15 mmol/l (Fig. 6). Relative to the amount of insulin secreted at 15 mmol/l glucose, further increases of 320, 77, and 60% were achieved by the inclusion of 100 µmol/l of isobutylmethylxanthine (IBMX), 1 mmol/l oleate/palmitate (2:1 molar ratio), or 50 nmol/l glucagon-like peptide 1 (GLP-1), respectively. In contrast to the clear effects of these agents, glucose-stimulated insulin secretion was not potentiated by the inclusion of a muscarinic receptor agonist (100 µmol/l carbachol) (Fig. 6). The lack of effect of carbachol on INS-1 cells is consistent with our previous studies and contrasts with other rat cell lines such as RIN1046-38 and its derivatives, which exhibit a potent carbachol response (21).

K_{ATP} channel-dependent and -independent pathways of insulin secretion in the 832/13 INS-1 cell line. We next investigated the involvement of the K_{ATP} channel in the glucose response of line 832/13 by performing experiments in the presence of a sulfonylurea (tolbutamide), which interacts with the sulfonylurea receptor to cause channel closure, and diazoxide, an opener of the channel. As shown in Fig. 7, treatment of 832/13 cells with 250 µmol/l diazoxide completely blocked the stimulation of insulin secretion caused by

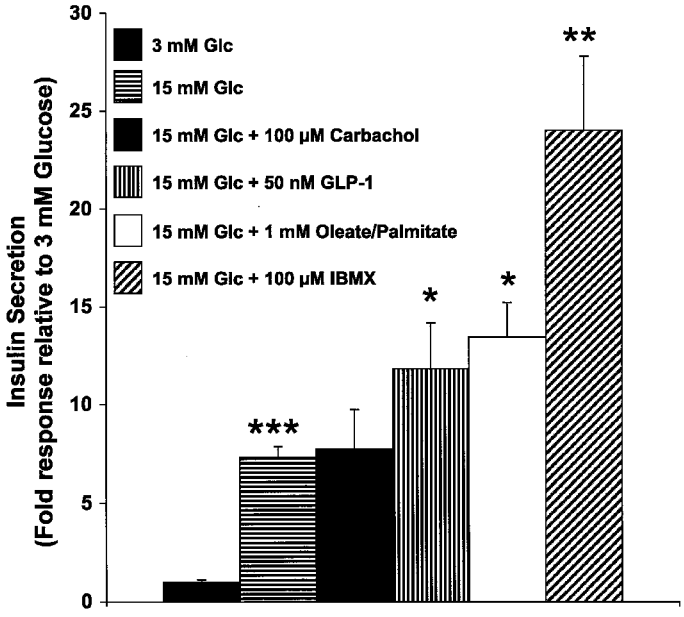

FIG. 6. Potentiation of glucose-stimulated insulin secretion in the INS-1–derived clone 832/13. The 832/13 cells were incubated in low (3 mmol/l) or high (15 mmol/l) glucose (Glc) in the presence and absence of known potentiators of insulin secretion as indicated in the legend. Data represent the means ± SE for five independent experiments. Paired two-tailed analyses were performed by comparing the 15 and 3 mmol/l glucose groups or by comparing 15 mmol/l glucose + potentiator groups with 15 mmol/l glucose alone. *P < 0.05; **P < 0.01; ***P < 0.001.

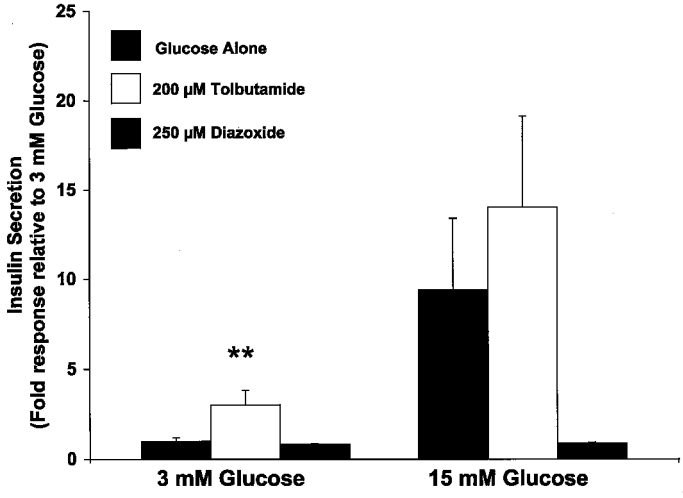

FIG. 7. Evidence for operation of the K_{ATP} channel system in the INS-1–derived clone 832/13. The 832/13 cells were incubated at 3 or 15 mmol/l glucose in the presence or absence of 200 µmol/l tolbutamide or 250 µmol/l diazoxide. Data represent the means ± SE for six independent experiments. **Insulin secretion in the 3 mmol/l glucose + tolbutamide-treated cells was greater than in cells treated with 3 mmol/l glucose alone (P < 0.01).

15 mmol/l glucose. In contrast, 200 µmol/l tolbutamide stimulated insulin secretion by threefold at 3 mmol/l glucose and by 50% at 15 mmol/l glucose, although the effect at high glucose did not quite reach statistical significance. The lesser effect of tolbutamide at high glucose is consistent with the very effective action of glucose alone on K_{ATP} channel closure.

In addition to the K_{ATP}-channel-dependent pathway, glucose has been shown to regulate insulin secretion from normal islets in which the K_{ATP} channel was bypassed by treatment with depolarizing K^+ (22,23). We investigated whether this K_{ATP} channel-independent pathway was operative in our new cell lines.

Figure 8 shows insulin secretion from parental INS-1 cells, a representative "poorly responsive" transfected INS-1 clone (834/105), and the "strongly responsive" 832/13 cell line in the presence of 35 mmol/l K^+. This high concentration of K^+ depolarizes the plasma membrane directly, circumventing the requirement for glucose-induced closure of K_{ATP} channels. Under these conditions, both the parental INS-1 cells and the poorly responsive clone exhibited less than a doubling of insulin secretion as glucose was increased from 3 to 15 mmol/l, and opening the K_{ATP} channels with diazoxide did not affect either the basal or stimulated insulin output as expected in the presence of depolarizing K^+. Compared with either of these populations, 832/13 cells exhibited much better K_{ATP} channel-independent signaling in that 15 mmol/l glucose stimulated insulin secretion by 4.5-fold relative to output at 3 mmol/l glucose; diazoxide was again without effect. We also studied the glucose concentration dependence for stimulation of insulin secretion from 832/13 cells by the K_{ATP} channel-independent pathway (in the presence of 35 mmol/l K^+ and diazoxide). We found that the concentration dependence was virtually identical in cells studied at 35 mmol/l K^+ (Fig. 9) compared with cells studied at 4.8 mmol/l K^+ (Fig. 5), although the fold stimulation of insulin secretion was less in the former than in the latter experiments. These data in combination with the experiments in Fig. 7 provide clear evidence of the presence of both K_{ATP} channel-dependent and -independent mechanisms of glucose-stimulated insulin secretion in 832/13 cells.

DISCUSSION

Dual motivations exist for developing INS cell lines that faithfully mimic the function of normal pancreatic islet β-cells. First, our understanding of the precise biochemical mechanisms of fuel-stimulated insulin secretion has developed relatively slowly because of the difficulty inherent in isolating fully functional pancreatic islets from humans or animals. In addition, secretory responses to glucose and its potentiators begin to wane within hours of islet isolation and maintenance in tissue culture. Thus, a cell line that stably responds to physiologically relevant secretagogues could be valuable for defining mechanisms involved in the regulation of insulin secretion, which may possibly lead to the development of new pharmaceutical reagents for treating diabetes. Second, cell lines that faithfully mimic the function of normal pancreatic islets could serve as surrogates for islets in transplantation therapy for type 1 diabetes (25,26). To fulfill this role, candidate cell lines must secrete insulin over the physiological range of glucose concentrations and should also be responsive to known potentiators of glucose signaling. Most importantly, these responses must be stably maintained in both in vitro and in vivo settings. In the present study, we demonstrate that the commonly used INS-1 cell line consists of a mixture of glucose-responsive and -unresponsive cells. We further show that stable transfection of parental INS-1 cells and isolation of discrete colonies result in new cell lines with markedly improved function compared with the original pop-

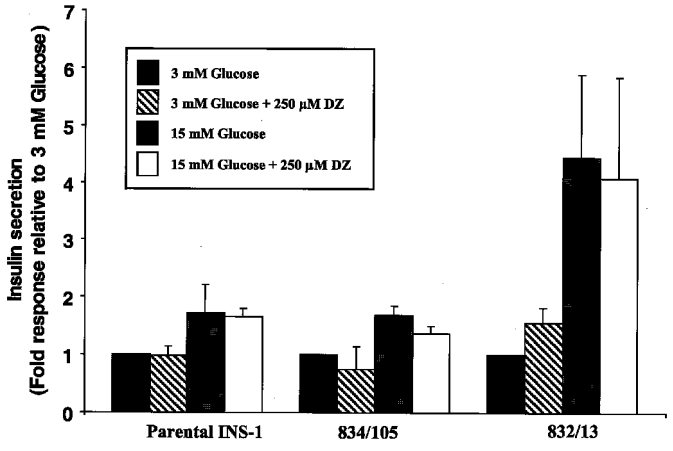

FIG. 8. Improved K_{ATP} channel-independent pathway of glucose-stimulated insulin secretion in INS-1–derived clones. Insulin secretion from parental INS-1 cells, a representative clone poorly responsive to glucose (834/105), and a representative clone strongly responsive to glucose (832/13) was measured in the presence of a depolarizing concentration of K^+ (35 mmol/l), which provides a measure of K_{ATP} channel-independent glucose signaling. Secretion was measured in cells exposed to low (3 mmol/l) or high (15 mmol/l) glucose in the presence and absence of 250 μmol/l diazoxide (DZ) as indicated in the legend. Insulin secreted into the medium was measured with RIA, was normalized to total cellular protein, and was expressed as fold increase relative to insulin secreted at 3 mmol/l glucose. Data represent the means ± SE for three independent experiments.

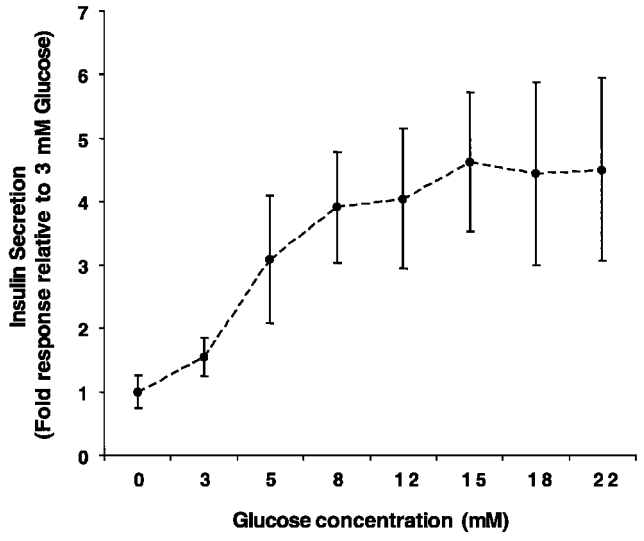

FIG. 9. Glucose dose-response curve for K_{ATP} channel-independent secretion from INS-1–derived clone 832/13. The 832/13 cells were incubated at the indicated concentrations of glucose for 2 h in the presence of 35 mmol/l K^+ and 250 μmol/l diazoxide to allow measurement of K_{ATP} channel-independent signaling. Insulin secreted into the medium was measured with RIA, was normalized to total cellular protein, and was expressed as fold increase relative to insulin secreted at 3 mmol/l glucose. Data represent the means ± SE for four independent experiments.

ulation. The enhanced secretory responsiveness to glucose is maintained for at least 7.5 months in tissue culture, which suggests that the newly derived lines may be a useful new tool for studying the mechanisms of insulin secretion and preclinical transplantation studies in animal models of diabetes.

The apparent clonal heterogeneity of the original INS-1 cell line is understandable in the light of the methods used for its isolation. Asfari et al. (6) dispersed cells from transplantable X-ray–induced INS tumors from NEDH rats and then separated viable cells from aggregates and debris by Percoll gradient centrifugation. The resultant preparation of cells was cocultured with lymphocytes in the presence of β-mercaptoethanol, which was added to enhance survival and proliferation of lymphocytic cells. INS-1 cells were isolated as free-floating cell aggregates from these coculture experiments. Based on this description of methods, such aggregates may have contained cells of different origin and degree of differentiation.

In addition to robust glucose responsiveness, the new INS-1 cell lines isolated with stable transfection exhibit other attractive features. First, the glucose effect is potentiated effectively by agents known to enhance glucose-stimulated insulin secretion from normal pancreatic islets such as IBMX, GLP-1, free fatty acids (a 2:1 oleate/palmitate mixture), and the sulfonylurea tolbutamide. Second, in the presence of physiological K^+ concentrations, the new lines exhibit a complete inhibition of insulin secretion by the K_{ATP} channel opener diazoxide, which indicates that regulation of this channel is an important component of their glucose response, as is the case in normal islets. Third, the new lines have a much more pronounced K_{ATP} channel-independent glucose-sensing pathway than parental INS-1 cells such that the new cells have responses similar to those noted in normal islets by Gembal et al. (22). These findings suggest that the new lines will be improved models for studying key issues in β-cell stimulus/secretion coupling. For example, our recent study showing a lack of effect of a malonyl CoA decarboxylase adenovirus or the drug triacsin C on glucose-stimulated insulin secretion argues against the long-chain acyl CoA hypothesis of glucose sensing (12). However, these studies can be justifiably criticized because the parental INS-1 cells used exhibited only 2- to 4-fold responses to glucose, which are well below the 15-fold response of normal islets. Furthermore, based on the work described herein, we now know that parental INS-1 cells have a very limited K_{ATP} channel-independent pathway of glucose sensing, and regulation by long-chain acyl CoAs could occur primarily via this pathway. The robust glucose-stimulated K_{ATP} channel-dependent and -independent responses coupled with the clear potentiating effect of free fatty acids suggest that the new cell lines will be useful for more rigorous and relevant testing of this and other models in the future.

The new 832/13 cell line differs in some fundamental respects from normal islets. First, the cells begin to respond to glucose at a slightly lower threshold concentration (4 mmol/l) than normal rat islets (5.5 mmol/l) and are also maximally responsive at 8 mmol/l glucose, whereas normal rat islets continue to respond to concentrations of ≥15 mmol/l. Second, unlike normal islets, 832/13 cells do not exhibit potentiation of glucose-stimulated insulin secretion by carbachol, a muscarinic receptor agonist. One possible explanation for the shift in glucose responsiveness in 832/13 cells relative to normal islets could be the use of RPMI medium containing 11.1 mmol/l glucose to grow the cell lines used in this study. Thus, culturing parental INS-1 cells (27) or human pancreatic islets (28) at elevated glucose levels

(11–25 mmol/l) causes a modest left shift in glucose dose dependence for insulin secretion. Possible mechanisms by which a left shift may occur include a change in the ratio between glucokinase and low K_m hexokinases (27) or altered expression of more global factors such as HNF-1α. A recent study has shown that mice with heterozygous knockout of HNF-1α have a slightly left-shifted glucose dose-response profile compared with normal islets, although islets from the HNF (+/–) animals did maintain responsiveness at glucose concentrations >8 mmol/l (29). The lack of responsiveness to carbachol is more difficult to explain. We have previously shown that carbachol effectively elevates inositol phosphates in INS-1 cells, which provides evidence that muscarinic receptor signaling is normal in these cells (21). Perhaps INS-1 cells have low or absent levels of expression of the atypical protein kinase C isozyme (ζ) that has been implicated in carbachol-stimulated insulin secretion in RIN cells (30).

Further investigation will be necessary to understand the mechanisms underlying the differences in phenotype between the new INS-1 lines and normal rat islets. Nevertheless, the INS-1–derived cell lines described herein appear to be an attractive new tool for investigating metabolic signaling mechanisms in islet β-cells. Future studies will focus on understanding the molecular mechanisms responsible for the subtle differences in glucose responsiveness between the new INS-1 cell lines and normal rat islets and the more pronounced differences between the strongly responsive and poorly responsive INS-1 cell clones.

ACKNOWLEDGMENTS

The work was supported in part by National Institutes of Diabetes and Digestive and Kidney Diseases Grant DK-42582 and a National Institutes of Health/Juvenile Diabetes Foundation International Research Program Grant (C.B.N.) and grants from the Medical Research Council of Canada, the Canadian Diabetes Association, and the Juvenile Diabetes Foundation International (M.P.). In addition, H.M. received fellowship support from the Juvenile Diabetes Foundation International, and G.C. received partial support from NIH Training Grant T-32-GM-08203.

The authors thank Drs. Roger Unger and Anice Thigpen for critical reading of the manuscript and Teresa Eversole for expert technical assistance.

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