Pancreatic β Cell Heterogeneity in Glucose-induced Insulin Secretion*

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Rat pancreatic β cells differ in their individual sensitivity to glucose-inducible metabolic changes. The present study examines whether β cells with a higher metabolic threshold require higher glucose levels for stimulation of their secretory activity. Purified β cells were distributed according to their metabolic redox state at 7.5 mM glucose; the metabolically responsive (high responsive) and unresponsive (low responsive) subpopulations of comparable size and viability were reaggregated in the presence of [3H]tyrosine and then perfused at 2.8 mM glucose with 10-min pulses of increasing glucose concentration. Glucose elicited first-phase insulin release in both high and low responsive subpopulations, whereas it increased dose-dependently in the low responsive one. These results indicate the existence of intercellular differences in the secretory activity of glucose-exposed β cells, both in terms of glucose sensitivity and of amplitude. This heterogeneity in β cell secretory responsiveness parallels that which has been previously described for the cellular metabolic and biosynthetic functions. It is concluded that glucose dose-dependently recruits β cells into both biosynthetic and secretory activities. Co-existence of inactive and activated cells can explain preferential release of newly synthesized over preformed hormone during glucose stimulation.

In vitro studied rat β cells have been found to differ in their individual sensitivity for glucose (1-5). The cells exhibit differences in their individual redox state at low, intermediate, and high glucose concentration (1, 5). An increase in glucose results in a higher percentage of cells with a reduced metabolic redox state (1, 5). Cells which require higher glucose levels for such metabolic shift are characterized by a higher threshold for glucose-induced biosynthetic activity (5). The present study examines whether a similar correlation exists between the metabolic responsiveness of the cells and their glucose-inducible secretory activity. This question is examined in purified β cell subpopulations in which the existence of cellular heterogeneity can be more directly assessed (1, 5) than in unpurified islet cell preparations (2, 4). This model can also neglect interference from other islet cell types which are known to influence hormone release from islet β cells in vitro (6), and thus alter individual β cell responses to glucose. Since heterogeneity in the pancreatic β cell population may be responsible for the preferential release of newly formed insulin (7-10), we compared β cell responses in terms of the released contents of preformed as well as of newly formed hormone.

EXPERIMENTAL PROCEDURES

Purification of Islet β Cells—Islets of Langerhans were isolated from male adult Wistar rats after collagenase digestion of the pancreas (11). Isolated islets were dissociated in a calcium-free medium containing trypsin (Boehringer Mannheim; 25 μg/ml), DNase (Boehringer Mannheim; 2 μg/ml), and 1 mM EDTA (11). Islet β cells were purified by autofluorescence-activated cell sorting (PACS Star, Beckton Dickinson, CA) using cellular light-scatter and FAD-autofluorescence as discriminating parameter (11). In the first series of experiments, isolated β cells were immediately labeled with [3H]tyrosine and tested for their secretory responsiveness (see further). In the second set, purified β cells were first distributed according to their metabolic responsiveness before being labeled and tested.

Preparation of Islet β Cell Subpopulations Differing in Metabolic Responsiveness—Isolated β cell preparations were resuspended in Earle's Heps buffer (for composition see Ref. 11) containing 1% BSA (fraction V, RIA grade, Sigma) and 7.5 mM glucose, and then incubated for 15 min at 37 °C. The cells were again submitted to autofluorescence-activated cell sorting, this time using their NAD(P)H autofluorescence and light scatter as discriminating parameter (1, 5). In this condition, approximately 50% of the cells exhibit higher than basal NAD(P)H levels allowing their separation by FACS sorting (6). The remaining cells were recovered in the window of basal NAD(P)H levels (6). Both the low NAD(P)H subpopulation (low responsive) and high NAD(P)H subpopulation (high responsive) were counted in a Bürker chamber and tested for their viability by neutral red staining. Only preparations with more than 90% neutral red positive cells were used for further functional analysis, which was the case for 16 out of 18 independent experiments.

Peptide Labeling in Isolated Islet β Cell Preparations—Purified β cell subpopulations were washed and suspended in HAM's F-10 medium supplemented with 7.5 mM glucose, 1% BSA, 2 mM L-glutamine and 50 μM 3-isobutyl-1-methylxanthine. They were then reaggregated during a 90-min shaking incubation in Lux dishes containing 25 or 50·10^6 cells in 3 ml of medium (6). The last 60 min of this incubation occurred in the presence of 5 μl·L^-1·[3,5-3H]tyrosine (TRK 200, Amersham, Bucks, United Kingdom; 2.10^12 Bq/mmol). After washing the cells in HAM's F-10 containing 2.8 mM glucose and 1 mM L-tyrosine, samples were taken for insulin assay (6) and for viability testing (6) and the remaining material used for perfusion. The time period between the end of the labeling incubation and the start of perfusion was kept between 30 and 45 min.

Batch Incubation—Washed [3H]tyrosine-labeled cells were resus-
pendent in HAM’s F-10 supplemented with 1% BSA, 2 mM L-glutamine, and 50 μM 3-isobutyl-1-methylxanthine and containing either 6.1 or 20 mM glucose. Groups of 5.10^6 β-cells were distributed over multiwell cups (1.5 ml; Nunclon Giboco) and statically incubated in a CO₂ incubator. After 2, 4, or 18 h of incubation, all cells were removed from the wells, centrifuged, and the supernatant assayed for total and [³H]labeled insulin immunoreactivity. Glucose-induced hormone release was calculated as the difference between medium insulin content at 20 mM and that at 6.1 mM glucose. The pelleted cells were extracted in 2 M acetic acid, 0.25% BSA for determining their content in total and [³H]-labeled (pro)insulin. Microtiter-plated cells were also incubated in parallel for determining their viable staining at the end of the study period.

Insulin Release during Perfusion—A multiple microchamber module (Endotronics, Coon Rapids, MN) with pump and thermostat was used for perfusion of the [³H]-labeled β-cell preparations (10). The cell preparations were loaded on preformed Bio-gel P2 columns (Bio-Rad) and perfused with HAM’s F-10 equilibrated with 95% O₂/5% CO₂. The cells were first exposed to 2.8 mM glucose till min 30 and then to a sequence of 10-min pulses of increasing glucose concentration, which alternated with 10-min phases at 2.8 mM glucose. At a flow rate of 1 ml/min, samples were collected over 1 min and assayed for immunoreactive insulin. For each glucose pulse, the three samples with the highest insulin content were pooled and assayed for [³H] protein, [³H]labeled insulin, and total insulin (10). At the end of each perfusion, the cells were extracted in 2 M acetic acid, 0.25% BSA and assayed for total and [³H]-labeled (pro)insulin and for total [³H]protein (10).

A database was constructed in the Statview program on Apple Macintosh Plus. Unless otherwise stated, significance of differences was calculated by two-tailed paired Student’s t testing. Part of the data was processed according to the Excel 2.2 program.

RESULTS

Glucose-induced Insulin Release from Reaggregated β Cells during 18-h Culture—The glucose-induced increase in medium hormone content was determined for the 0–2, 2–4, and 4–18-h intervals and expressed as nanograms of insulin or cpm of [³H]insulin released per h of culture. Total immunoreactive insulin release was highest during the first 4 h of culture, with a 3–4-fold lower mean rate during the 4–18-h interval (p < 0.05, Table I). In terms of [³H]-labeled insulin release, glucose stimulation was most potent during the first 2 h of culture, decreasing to 20-fold lower rates during the 4–18-h period (p < 0.005, Table I). The different release patterns of newly synthesized and preformed insulin are reflected in the decreasing ratio of released [³H]insulin over released insulin (Table I). Comparison of these specific radioactivities of released insulin with the specific radioactivity of cellular insulin indicates that the newly synthesized hormone is preferentially released during the first 4 h of culture (p < 0.01, Table I).

Dose Responsiveness of Glucose-induced Insulin Release from Reaggregated β Cells—The dose responsiveness of glucose-induced insulin release from reaggregated B cells was determined in perfusion. As reported previously (10), 0.01% of cellular insulin was released per min at low glucose (2.8 mM (Fig. 1)); a similar fraction of the cellular [³H]-labeled insulin pool was discharged under this condition (Ref. 10 and Fig. 2). The specific radioactivity of the released hormone was comparable to that measured in the cells, i.e. ± 200 cpm/ng insulin (Fig. 3). When the glucose level of the medium was increased to 4.2 mM, a phasic release of insulin was noticed with peak values around min. 4 (Fig. 1). When the three peak fractions were pooled and assayed, immunoreactive and [³H]-labeled insulin release were found to be increased by, respectively, 60 and 270% (p < 0.01; Fig. 2). The specific radioactivity of insulin released at 4.2 mM was 2-fold higher than that in the medium at 2.8 mM (p < 0.001) and that in the cellular hormone store at start (p < 0.001) (Fig. 3).

The amplitude of glucose-induced hormone release increased dose dependently (Fig. 1). At 8.3 mM glucose, the pooled fraction contained 130% more immunoreactive insulin than at 4.2 mM (p < 0.02, Fig. 2). A further 30% increase occurred between 8.3 and 22.2 mM glucose (p < 0.05, Fig. 2). The initiation of a second phase insulin release was detected from glucose levels of 8.3 mM on (Fig. 1).

Compared to the release of total insulin, that of [³H]-labeled insulin rose more rapidly at the lower glucose levels, reaching its maximum at 8.3 mM glucose (Fig. 2). That the release of newly synthesized insulin exhibits a different glucose sensitivity than that of total insulin is clearly reflected by the glucose-dependent variation in specific radioactivity of secreted insulin (Fig. 3). This ratio of [³H]insulin over total insulin rises 3-fold between 2.8 and 5.6 mM glucose and then progressively declines without returning to the initial values (Fig. 3).

Comparison of Glucose-induced Insulin Release from Islet β Cell Subpopulations Separated on the Basis of Metabolic Responsiveness to 7.5 mM Glucose—Parallel perfusions compared the secretory responsiveness of two islet β cell subpopulations which had been separated on the basis of their positive and negative metabolic responses to 7.5 mM glucose. The two preparations were composed of similar numbers of β cells at a similar degree of purity and viability. Total cellular insulin content did not differ (p > 0.05), but 2-fold more [³H]-labeled insulin was measured in the metabolically responsive subpopulation (Table II): the specific radioactivity of the stored hormone was thus 2-fold higher in the responsive preparation than in the unresponsive one (Table II).

At 2.8 mM glucose, both subpopulations discharged 0.01% of their initial hormone stores per min (Fig. 4). Exposure to 4.2 or 5.6 mM glucose elicited a phasic insulin release in the responsive subpopulation but not in the unresponsive one (Fig. 4).

### Table I

<table>
<thead>
<tr>
<th>Glucose-induced insulin release per h of</th>
<th>0-2 h</th>
<th>2-4 h</th>
<th>4-18 h</th>
<th>Cellular hormone content before start of culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (ng)</td>
<td>55 ± 6^a</td>
<td>91 ± 17^a</td>
<td>26 ± 6</td>
<td>1848 ± 170</td>
</tr>
<tr>
<td>[³H]insulin (10^8 cpm)</td>
<td>21.7 ± 2.8^a</td>
<td>11.4 ± 3.7^a</td>
<td>0.8 ± 0.3</td>
<td>212 ± 24</td>
</tr>
<tr>
<td>[³H]insulin/insulin (cpm/ng)</td>
<td>350 ± 14^a</td>
<td>246 ± 19^a</td>
<td>107 ± 13</td>
<td>114 ± 3</td>
</tr>
</tbody>
</table>

[^a]: S.E. of four independent experiments. Paired Student’s t test versus [³H]insulin released between 4 and 18 h: *p < 0.05; †p < 0.02; ‡p < 0.005; ¶p < 0.05 (one-tail). Paired Student’s t test versus ratio in cellular hormone store: *p < 0.001; †p < 0.01. Paired Student’s t test versus ratio in medium collected at the end of the 4-h period, *p < 0.05.
From 8.3 mM on, the unresponsive subpopulation was stimulated to glucose-induced insulin release with a dose-dependent increase up to the maximal tested dose of 22.2 mM (Fig. 4). The rates of hormone release remained however 2-fold lower than in the responsive subpopulation (p < 0.001, Fig. 4).

The two β cell subpopulations also differed in their respective rates of 3H-labeled insulin release during glucose stimulation. At 2.8 mM glucose, both preparations released 0.01% of their 3H-labeled insulin content (Fig. 5). Exposure to 4.2 mM glucose increased the rate of 3H-insulin release to 0.08 ± 0.01% per min in the responsive subpopulation (p < 0.005), but only to 0.03 ± 0.002% per min in the unresponsive subpopulation (p < 0.01). At 8.3 mM glucose, the release of 3H-insulin was maximal in the responsive cells (0.14 ± 0.005% of content released per min) and half-maximal in the unresponsive ones (0.07 ± 0.01% of content). At 22.2 mM glucose, both subpopulations released similar fractions of their 3H-labeled insulin pool (Fig. 5). In absolute terms however, the metabolically responsive subpopulation, which contained 2-fold more 3H-labeled insulin per 10^6 cells (Table II), secreted 2-fold more newly formed hormone per 10^6 cells.

In both subpopulations, glucose was found to induce preferential release of newly synthesized hormone. In the metabolically responsive subpopulation, this effect was already maximal at 4.2 mM glucose, where the release of newly formed hormone occurred at 4-fold higher rates than that of preformed hormone. This was also the case during subsequent pulses at 5.6 and 8.3 mM glucose, whereas stimulation at 11.1 and 22.2 mM glucose was associated with a lower degree of preferential release (Fig. 3). In the unresponsive subpopulation, the preferential release of newly formed hormone increased progressively between 2.8 and 11.1 mM glucose (Fig. 3); the maximal glucose effect consisted also of a 4-fold higher release of newly synthesized than of preformed insulin (Fig. 3).

**DISCUSSION**

In order to examine whether pancreatic β cells are heterogeneous in terms of their individual glucose-induced secretory activity, we choose to perfuse β cell (sub)populations shortly after their isolation. Use of purified β cells excludes interference by other islet types (11), analysis shortly after their isolation by sorting allows comparison of subpopulations with...
The three islet β cell preparations were reaggregated and labeled as described under “Experimental Procedures.” Cellular insulin and [3H]insulin content were determined before perfusion and expressed as the total amounts that were loaded on the perfusion columns. The specific radioactivity of the cellular insulin store was derived from these values. Data represent mean ± S.E. of six independent experiments. Statistical significance was determined by unpaired Student’s t tests: * p < 0.001 versus unresponsive subpopulation; ** p < 0.05 (one-tail) versus total population; *** p < 0.02 versus total population.

<table>
<thead>
<tr>
<th>Glucose (mM)</th>
<th>2.8</th>
<th>4.2</th>
<th>8.3</th>
<th>11.1</th>
<th>22.2</th>
<th>2.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total β cell population</td>
<td>8,133 ± 646</td>
<td>1,588 ± 179</td>
<td>7,500 ± 160</td>
<td>10,016 ± 1,164</td>
<td>127 ± 17</td>
<td></td>
</tr>
<tr>
<td>Subpopulation with metabolic response to 7.5 mM glucose</td>
<td>10,665 ± 1,544</td>
<td>2,591 ± 360</td>
<td>11,565 ± 1,780</td>
<td>248 ± 14^b</td>
<td>14 ± 1^c</td>
<td></td>
</tr>
<tr>
<td>Subpopulation without metabolic response to 7.5 mM glucose</td>
<td>11,016 ± 1,164</td>
<td>1,333 ± 97</td>
<td>127 ± 14^c</td>
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**TABLE II**

Comparison of pancreatic β cell subpopulations for their relative contents of stored and newly formed insulin.

The three islet β cell preparations were reaggregated and labeled as described under “Experimental Procedures.” Cellular insulin and [3H]insulin content were determined before perfusion and expressed as the total amounts that were loaded on the perfusion columns. The specific radioactivity of the cellular insulin store was derived from these values. Data represent mean ± S.E. of six independent experiments. Statistical significance was determined by unpaired Student’s t tests: * p < 0.001 versus unresponsive subpopulation; ** p < 0.05 (one-tail) versus total population; *** p < 0.02 versus total population.

**FIG. 4.** Insulin release from aggregates of the 7.5 mM responsive (lower panel) or unresponsive (top panel) β cell subpopulation during perfusion at the indicated glucose concentrations. Results are expressed as the percentage of the cellular hormone content that is released per min. Data represent mean ± S.E. of six independent experiments. Paired Student’s t tests versus the values measured at 2.8 mM glucose (for the 4.2 mM glucose pulse) or versus the peak values during the preceding glucose pulse (for all other glucose pulses) are: * p < 0.05; ** p < 0.02; *** p < 0.001.

**FIG. 5.** Release of preformed [3H]-labeled insulin during perfusion of the 7.5 mM responsive (□) and unresponsive (□) β cell subpopulation at the indicated glucose concentration. For each condition, the three samples with the highest insulin content first are pooled and analyzed for their [3H]insulin content. For the 2.8 mM condition, pooling was done for three samples immediately before the 4.2 mM glucose pulse. Release of [3H]insulin is expressed as a percentage of the cellular [3H]insulin content at start of perfusion. Data represent mean ± S.E. of six independent experiments. Paired Student’s t tests versus the values measured at 2.8 mM glucose (for the 4.2 mM glucose pulse) or versus the peak values during the preceding glucose pulse (for all other glucose pulses) are: *, p < 0.05; **, p < 0.01; ***, p < 0.005.

Different metabolic responsiveness to glucose (5), and perfusion of reaggregated cells yields potent biphasic secretory responses to glucose with a preferential release of newly formed hormone (10). In the present study, we exposed the purified β cell preparations to five 10-min pulses of increasing glucose concentration, which were separated by 10-min phases at low (2.8 mM) glucose. This perfusion protocol allowed detection of the cellular threshold for glucose-induced first phase release and an assessment of the dose responsiveness of its amplitude. The ratios of the released amounts of newly formed over preformed hormone were calculated to determine the degree of preferential release of newly synthesized insulin during each pulse. Previous studies on isolated islets have indicated that glucose stimulation leads to a preferential release of newly formed insulin as compared to preformed hormone (7-9). This finding has been interpreted as a possible expression of heterogeneity either within the secretory vesicles or within the pancreatic β cell population (7-9). The first form of heterogeneity implies favoring of newly formed hormone in its intracellular transport and subsequent release (7, 8), for example by marking their secretory vesicles (13), or by channeling through a constitutive pathway (14). The second form requires the existence of intercellular differences in the fractional release of newly formed total insulin (9, 10, 12). Comparison of β cell subpopulations with different metabolic sensitivity to glucose may indicate whether cellular heterogeneity can account for preferential release of newly formed hormone.

In purified β cell aggregates, first phase insulin release was already elicited by a 4.2 mM glucose pulse. Its amplitude increased during each of the successive pulses at increasing glucose concentration. It is not excluded that a glucose pulse may have primed the cells for a higher secretory activity during a subsequent stimulation (15). However, if such phenomena were to occur, they do not interfere with a comparative study of the glucose responsiveness in two β cell subpopulations.
After distributing the β cells according to their metabolic responsiveness at 7.5 mM glucose, we compared glucose-induced insulin release in two subpopulations of similar size and viability. The metabolically responsive subpopulation presented a secretory responsiveness that was comparable to that of the total population, i.e., a distinct first phase release during the 4.2 mM glucose pulse, a second phase appearing at the end of the 8.3 mM pulse, and a dose-dependent increase in the amounts of released hormone. In contrast, β cells which were metabolically unresponsive to 7.5 mM glucose did only exhibit secretory responses at glucose levels above 7.5 mM. Their response was biphasic and dose-dependent, but quantitatively inferior to that of the metabolically responsive subgroup despite the presence of a comparable insulin store. These data demonstrate that marked differences exist in the secretory activities of individual β cells, confirming observations in hemolytic plaque assays on unpurified islet cell preparations (2, 4). A strong correlation was found between the cellular metabolic responsiveness to glucose and the glucose-inducible secretory function. Taken together with previous findings (1, 3, 5), it is concluded that the intercellular differences in threshold for glucose-induced metabolic changes lead to cellular diversity in biosynthetic and secretory activities. The molecular basis for this heterogeneity has not yet been identified. The recognition of intercellular differences in glucokinase immunoreactivity (16) suggests that glucose phosphorylation should be considered as a possible regulatory site for β cell activity, as proposed by Matchinsky (17).

Under the present experimental conditions, the amount of insulin released between 2.8 and 8.3 mM glucose is primarily secreted by the subpopulation of cells which is metabolically responsive to 7.5 mM glucose. It is conceivable that the relative contribution of the other 40–50% β cells increases in the presence of other nutrient or hormonal stimuli. The possibility that a number of these β cells are at rest (4) or are involved in other functions such as cell division (18) needs also to be examined.

When the sorted subpopulation of metabolically unresponsive cells was incubated for 90 min at 7.5 mM glucose, its rate of insulin biosynthesis was half that in the metabolically responsive subgroup despite the presence of a comparable insulin store. The recognition of intercellular differences in glucose sensitivity of these cells differed markedly as evidenced by the respective dose-response curves for the release of newly formed hormone. These data confirm the existence of lower thresholds for glucose-induced insulin release in β cells with higher sensitivity to glucose-dependent metabolic changes. Preferential release of newly synthesized insulin was already maximally stimulated at 4.2 mM glucose in the metabolically sensitive subpopulation, whereas it increased progressively up to 11.1 mM glucose in the insensitive one. It can thus be concluded that preferential release of newly formed insulin is a consequence of intercellular differences in secretory activity. Cells activated for release will also be biosynthetically active, thus releasing preformed as well as newly formed hormone. This interpretation is compatible with recent observations using the hemolytic plaque assay showing intense biosynthetic labeling of more actively secreting β cells (19). Insulative cells contribute to the tissue pool of preformed hormone but not to its content in newly formed peptides. Since recruitment of β cells into glucose-induced activities is dose-dependent (3, 5), it is likely that the degree of preferential release of newly formed hormone varies with the glucose concentration. It can be speculated whether this particular feature can be used as an index for the state of functional heterogeneity in the pancreatic β cell population. This state of heterogeneity may be subject to physiologic variation, for example during aging (20), or pregnancy (21), two conditions associated with an altered glucose responsiveness of the pancreatic β cells.

The existence of intercellular differences in secretory activity has also been demonstrated in other cell types. Thyrotrophic cells, in a dose-dependent way, thyrocytes into secretion (22). Pituitary cells differ in their individual responsiveness to gonadotrophin-releasing hormone, thyrotrophin-releasing hormone, or estrogen (23-25). Cardiocytes are heterogeneous in their release of atrial natriuretic peptide (26). The notion that varying numbers of pancreatic β cells participate in the insulin secretory response to glucose may thus reflect a general feature of secreting hormone. In the perspective of a physiologic significance of β cell heterogeneity (27), the intercellular differences in glucose recognition may represent a key site in hormonal control of glucose homeostasis.

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REFERENCES