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Cell Biology

Glucose stimulates proinsulin biosynthesis by a dose-dependent recruitment of pancreatic beta cells

(protein synthesis/beta cell heterogeneity/insulin)

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ABSTRACT **Glucose is a well-known stimulus of proinsulin biosynthesis. In purified beta cells, the sugar induces a 25-fold increase in the synthesis of insulin immunoreactive material over 60-min incubation. Autoradiographic analysis of the individual cells shows that this effect is achieved via dose-dependent recruitment of pancreatic beta cells to biosynthetic activity. Recruitment of beta cells is also seen in isolated islets exposed to glucose. The sigmoidal dose–response curve for glucose-induced proinsulin biosynthesis thus reflects a heterogeneous responsiveness of pancreatic beta cells rather than a progressively increasing activity of functionally homogeneous cells. Dose-dependent recruitment of functionally diverse cells may be a ubiquitous mechanism in tissue function.**

The biosynthesis of proinsulin has been extensively studied in rat islet tissue (1–12). The process appears critically dependent on D-glucose (1, 3–5), which can regulate various steps in insulin gene expression by pancreatic beta cells (3, 7–12). Acute stimulation by the sugar has been located at the translational level (7, 8), whereas its chronic effects involve an action on the production (9–11) and/or stability (12) of preproinsulin mRNA. How individual pancreatic beta cells contribute to the overall biosynthetic process and its regulation is presently unknown. The heterogeneous and species-specific topography of beta cells has led to speculation on a functional diversity in this cell population (13, 14). *In vitro* studies on nonpurified (15, 16) and purified (17, 18) beta cell preparations support the coexistence of functionally heterogeneous beta cell subpopulations that can vary markedly in their response to glucose. The present study assesses this concept by comparing the glucose effects on protein synthesis in individual beta cells.

MATERIALS AND METHODS

Preparation of Islets and Purified Beta Cells. Islets of Langerhans were isolated from male adult Wistar rats (150–250 g) after collagenase digestion of the pancreas (19, 20). Cell suspensions were obtained by gentle dispersion of the islets in Ca^{2+}-free Earle's Hepes buffer (for composition, see ref. 20) supplemented with 1 mM EGTA, trypsin at 25 μg/ml (Boehringer Mannheim) and DNase at 2 μg/ml (Boehringer Mannheim) (21). Purified single islet beta cells were obtained by autofluorescence-activated cell sorting as has been described in detail in previous work from our laboratory (20, 22). The purity and viability of the isolated beta cell preparations exceeded 95% (20).

Labeling of Newly Synthesized Proteins. Samples of 5×10^4 beta cells or 50 islets were labeled in 0.2 ml of Earle's Hepes buffer containing 0.5% bovine serum albumin (fraction V, RIA grade, Sigma) and 50 μCi of L-[4,5-^{3}H]leucine (Amersham, Bucks, U.K.) (1 Ci = 37 GBq). The tracer was used at a concentration of 50 μM and a specific activity of 5 Ci/mmol, conditions found to saturate the biosynthetic process with exogenous leucine (unpublished observations). At end of the incubation the samples were diluted with 2.3 ml of Earle's Hepes buffer containing 2 mM unlabeled L-leucine. After extensive washing, cells were either extracted in 2 M acetic acid/0.25% bovine serum albumin for measurement of total protein and proinsulin biosynthesis or fixed and processed for autoradiography to assess the biosynthetic activity per cell.

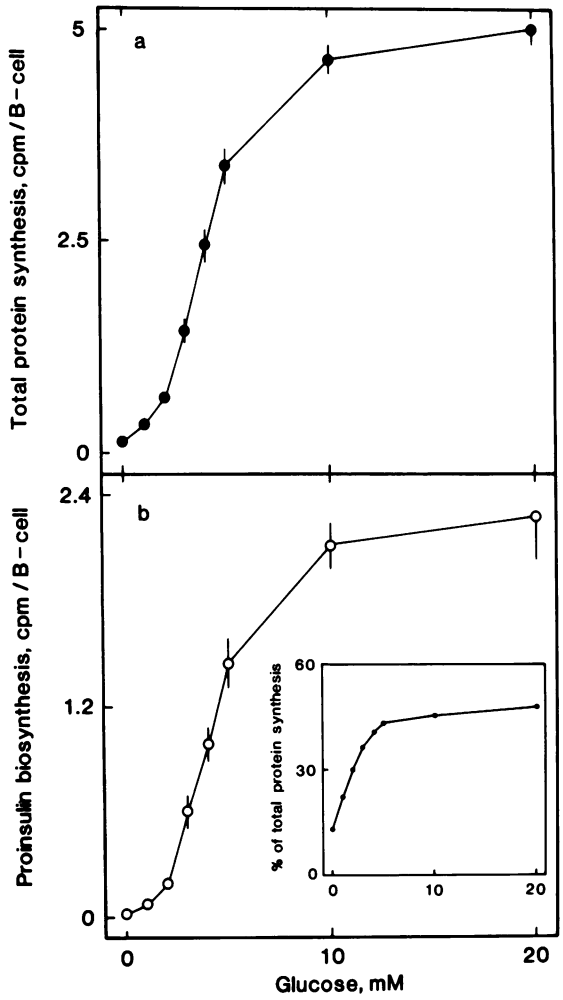

FIG. 1. Effect of glucose upon total protein synthesis (*a*) and proinsulin biosynthesis (*b*) by purified pancreatic beta cells (B-cell). Data are expressed as cpm per beta cell after correction for background radioactivity and represent mean values ± SEM ($n = 4$). (*Inset*) Effect of glucose (abscissa) on the ratio of proinsulin biosynthesis over total protein synthesis.

Total Protein and Proinsulin Synthesis. Samples suspended

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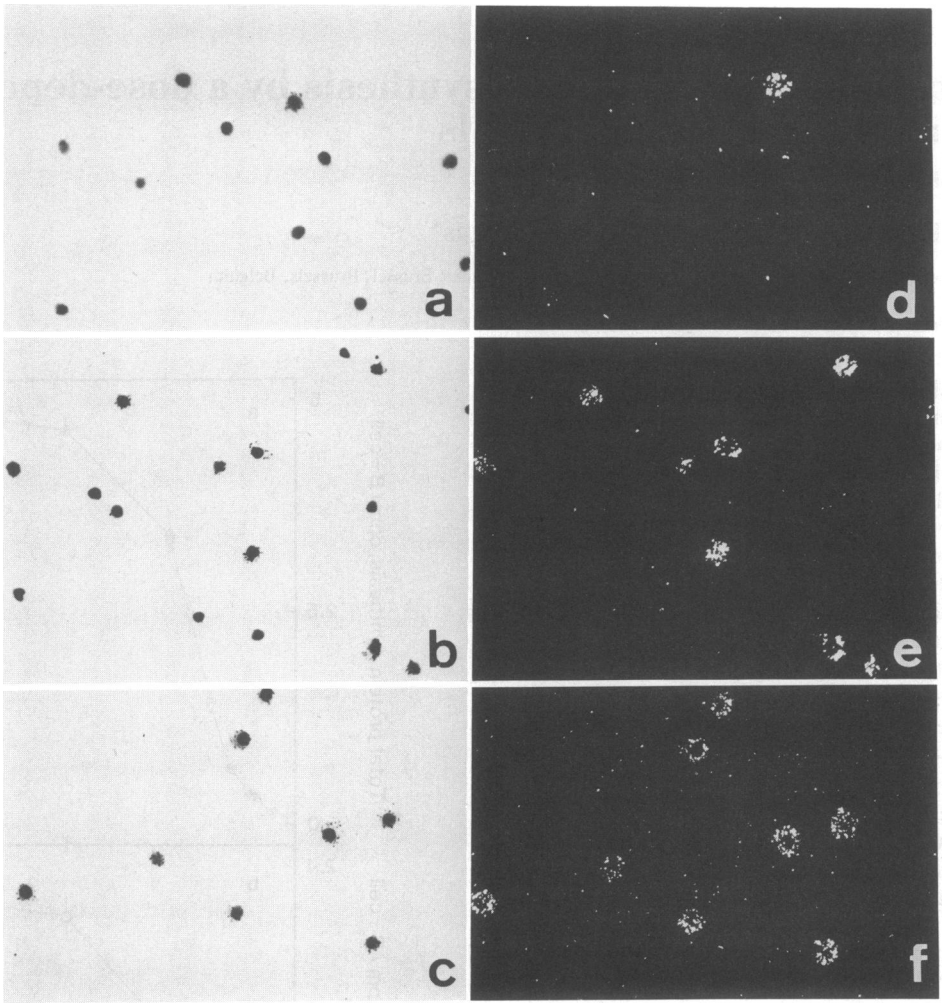

FIG. 2. Effect of glucose upon [^{3}H]leucine incorporation by individual beta cells. The figure shows the cells by transillumination (*a–c*) *and epipolarization microscopy* (*d–f*) after incubation at 1 mM (*a* and *d*), 3 mM (*b* and *e*), and 10 mM (*c* and *f*) glucose. The number of densely labeled cells increases with glucose concentration (*d–f*). (×200.)

in acetic acid were sonicated and centrifuged (1500 × *g* for 15 min at 4°C); the supernatant fraction was further analyzed. Total protein synthesis was measured as precipitable radioactivity after mixing 0.1-ml fractions of the acid extracts with 1.9 ml of 10% (vol/vol) Cl_3CCOOH (5). The Cl_3CCOOH pellets were solubilized in 1 M NaOH, neutralized with 1 M HCl, and counted after addition of 10 ml of Dynagel (Baker, Deventer, The Netherlands). Radioactivity in the Cl_3CCOOH supernatant was 13 ± 4% (mean ± SD; n = 30) of the total radioactivity in the acid extracts.

Proinsulin biosynthesis was measured as precipitable radioactivity after incubation of the cell extracts with excess anti-insulin serum. Fifty-microliter fractions of the acid extracts were dried *in vacuo* and redissolved in 0.2 ml of insulin assay buffer (phosphate-buffered saline with 0.5% bovine serum albumin, pH 7.6) containing 2.5 μl of undiluted guinea pig anti-porcine insulin serum (prepared by C. F. H. van Schravendijk from our laboratory). The samples were incubated for 60 min at 37°C, then mixed with 20 mg of protein A–Sepharose CL4B (Pharmacia, Uppsala) suspended in 0.2 ml of 0.9% NaCl (23, 24), again incubated for 15 min at room temperature, and finally centrifuged and extensively washed. The pellets were resuspended in 2 M acetic acid, and their soluble fraction was counted in 10 ml of Dynagel. Nonspecifically bound radioactivity was determined by incubating the samples with 2.5 μl of undiluted normal guinea pig serum instead of anti-insulin serum. Proinsulin biosynthesis was calculated as the difference between both counts. This difference exceeded the nonspecifically bound radioactivity by a factor of at least 6 and the counter background by a factor of 20. Recovery of insulin immunoreactive material was 95%, as assessed by running samples containing HPLC-purified ^{125}I-labeled insulin (25) in parallel. The specificity of the insulin antibody was demonstrated by the inability of an excess glucagon, somatostatin, or pancreatic polypeptide (250 ng/ml) to displace its binding to ^{125}I-labeled insulin. Under these conditions, glucose stimulation of proinsulin biosynthesis could be detected in samples of 1000 pancreatic beta cells.

Autoradiography of Beta Cells and Isolated Islets. After the purified beta cells were fixed in paraformaldehyde [2% in phosphate-buffered saline (PBS) for 15 min at room temperature] they were washed in PBS and dried on poly(L-lysine)-coated glass slides, postfixed in 2% glutaraldehyde (5 min at room temperature), washed again, and finally exposed for 4 hr at 4°C to autoradiographic L-4 emulsion (Ilford, U.K.). After being developed in Ilford ID-11 (6 min at 20°C) and fixation (2 min at 20°C) in Hypam (Ilford) the cells were counterstained with hematoxylin. Autoradiographic silver grains were counted by epipolarization microscopy at a final magnification of 200×. Background labeling—determined in parallel experiments without tracer—corresponded to 0.5 ± 1.4 grains per cell (mean ± SD). Therefore, only the cells with five or more grains per cell (above mean background value plus 3 SD) were scored as positive. In each tracer experiment, a grain count was done on 125 cells. The results

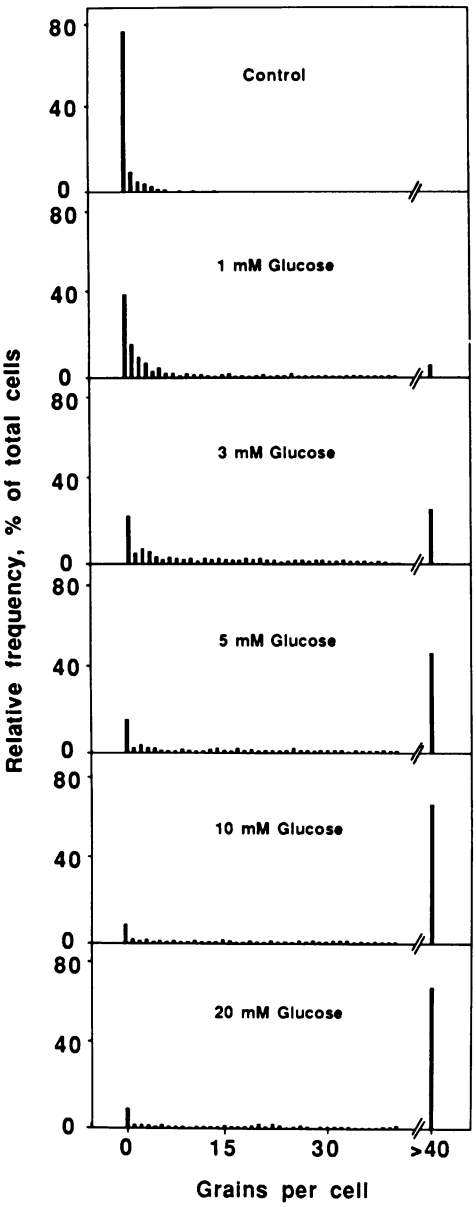

FIG. 3. Histogram of the number of autoradiographic silver grains per cell, plotted for different glucose concentrations. Control was done without any radioactive label. Bars represent mean values calculated from four independent experiments.

from four independent experiments were plotted in a frequency-distribution histogram. As counting was no longer accurate for grain densities >40 per cell, cells with >40 silver grains were classified together as one category.

The [^{3}H]leucine incorporation in individual beta cells was also visualized in collagenase-isolated islet tissue. The islets were fixed with 2.5% glutaraldehyde, postfixed in 1% OsO_4, and embedded in Spurr's resin before 1-μm sections were prepared. Sections were etched with sodium methoxide and stained for insulin using a guinea pig anti-insulin serum and a peroxidase-labeled anti-guinea pig serum. After coating the stained sections with a Formvar layer, the autoradiographs were exposed for 2 days and examined for silver grains on insulin-containing islet cells.

RESULTS

Glucose-Induced Protein Biosynthesis in Purified Beta Cells. The rate of protein biosynthesis by the total beta cell population depended markedly on glucose concentration

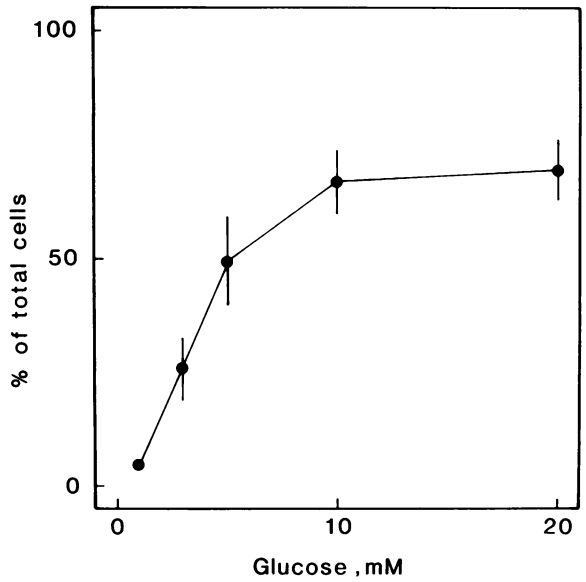

FIG. 4. Effect of glucose upon the percent of cells with >40 silver grains per cell. Data represent mean values ± SEM of four experiments.

during the labeling period (Fig. 1). The Cl_3CCOOH-precipitable radioactivity increased 13-fold when glucose was raised from 1 to 10 mM, but no further increment was measured at higher sugar levels (Fig. 1*a*). The dose–response curve of glucose-induced protein synthesis was sigmoidal in shape with half-maximal effect at 4 mM glucose. A similar dose dependency was noted for proinsulin biosynthesis, which was stimulated 25-fold when glucose increased from 1 to 10 mM (Fig. 1*b*). In the absence of glucose, 13% of labeled protein corresponded to insulin immunoreactive material. Raising the glucose concentration to 5 mM progressively increased this ratio to 40–50%, a range that was also measured at higher glucose levels (Fig. 1*b Inset*).

Biosynthetic Activity of Individual Beta Cells. Autoradiography of [^{3}H]leucine-labeled beta cells indicated that not all beta cells incorporated the amino acid at the same rate when exposed to the same glucose concentration (Fig. 2). At 1 mM glucose, only few cells were heavily labeled, at 3 mM almost half of them appeared positive, whereas at 10 mM a large majority of the cells were intensely stained (Fig. 2). Counting the number of silver grains per cell demonstrated that glucose increased, in a dose-dependent manner, the percentage of positive cells (i.e., cells with a grain count higher than the mean background value plus 3 SD): 28 ± 7% at 1 mM, 59 ± 13% at 3 mM, 77 ± 6% at 5 mM, and 86 ± 5% at 10 mM glucose (mean values ± SD; n = 4). When cells were plotted according to their respective number of silver grains, glucose increased the fraction of beta cells with >40 grains per cell but not that fraction with 5–40 grains per cell (Fig. 3). Although at 1 mM glucose only 5 ± 1% of the cells belonged to the former category, this figure increased to 27 ± 14% at 3 mM, 48 ± 18% at 5 mM, and 68 ± 15% at 10 mM glucose. Increasing the glucose concentration from 10 to 20 mM did not further increase the percent positive beta cells ($P > 0.05$) nor the fraction of positive cells with >40 grains per cell ($P > 0.05$). From these observations, the glucose-induced recruitment of cells to biosynthetic activity can be expressed by a dose–response curve of the number of cells with >40 grains per cell (Fig. 4). The recruitment of cells is most prominent between 1 and 5 mM glucose, increasing almost linearly in this concentration range, and levels off between 5 and 10 mM (Fig. 4). The glucose-induced recruitment of pancreatic beta cells was also seen after longer incubation periods with glucose and

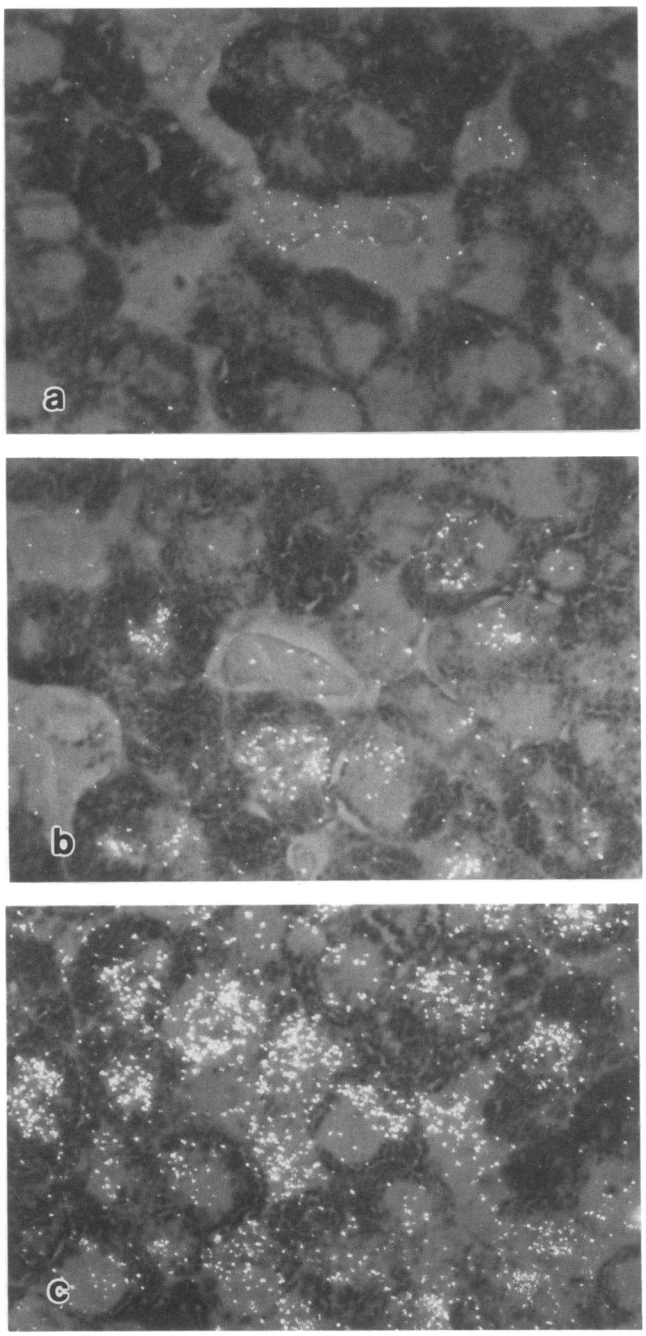

FIG. 5. Effect of glucose upon [^{3}H]leucine incorporation by individual beta cells in isolated islets. Autoradiographs of 1-μm islet sections are shown in epipolarization microscopy after immunostaining for insulin. Islets were incubated at 1 mM (*a*), 5 mM (*b*), and 10 mM (*c*) glucose. Glucose increased in a dose-dependent manner the percentage of radioactively labeled insulin-containing cells, i.e., those with silver (white) grains. (×1025.)

[^{3}H]leucine. After a 120-min exposure, the percentage of positive beta cells was 14 ± 6 at 1 mM, 42 ± 16 at 3 mM, and 76 ± 9 at 10 mM glucose (mean values ± SD; n = 4).

The phenomenon of cell recruitment was also present in undissociated islet tissue. At 1 mM glucose, only few intensely labeled beta cells were discerned, and most grains accumulated in noninsulin immunoreactive cells that were located at the periphery of the isolated islets. At 5 mM glucose, a distinct fraction of the insulin-containing beta cells had incorporated the radioactive amino acid, whereas the majority of the cells became positive at 10 mM glucose (Fig. 5).

DISCUSSION

As expected from previous studies on islet tissue (1–12) glucose appeared a potent stimulus of protein synthesis in pancreatic beta cells. The biosynthetic process was most sensitive to variations in glucose concentration between 2 and 5 mM—the range in fasting rats (10, 26)—and was maximally stimulated at 10 mM. The glucose regulation of proinsulin biosynthesis thus clearly differs from that of insulin release, which is triggered by glucose levels above 5 mM and still augmented beyond 10 mM (27, 28). These differences in glucose sensitivity may provide physiologically important properties to a healthy beta cell population as such differences may permit replacement of insulin stores during fasting and extra discharge of insulin in exceptional conditions of hyperglycemia. It is, however, unknown whether these particular glucose sensitivities represent a common feature of each pancreatic beta cell or whether the beta cell population is composed of cells that differ in the glucose sensitivity of their biosynthetic and/or secretory activity. In a model of beta cell homogeneity, all individual beta cells exhibit identical sigmoidal dose–response curves for the glucose-induced biological functions. In a model of beta cell heterogeneity, a sigmoidal dose–response curve of the total population expresses a dose-dependent recruitment of beta cells to a glucose-induced biological action. The autoradiographic analysis of [^{3}H]leucine-labeled cells allowed us to assess which model applied to glucose regulation of biosynthetic activity. Thus the increased rate of protein synthesis by a glucose-exposed beta cell population was shown to be the result of a progressively increasing number of beta cells with glucose-induced biosynthetic activity. This glucose-induced recruitment of cells is dose-dependent and most pronounced at sugar concentrations between 1 and 5 mM, the range where the rate of total protein synthesis is also most accelerated. This recruitment fades out between 5 and 10 mM, which further parallels total protein synthetic activity. These results clearly indicate that the pancreatic beta cell population is composed of cells which—at least *in vitro*—differ in their respective sensitivities to the stimulatory action of glucose. The results agree with earlier electrophysiological recordings in unpurified islet tissue (15) and more recent insulin release measurements in unpurified islet cell preparations (16, 29), which both suggested differences in glucose sensitivity of individual beta cells. Our observations also provide direct evidence for the concept of a functional heterogeneity within the pancreatic beta cell population (18).

The recognition of beta cell subpopulations with different glucose sensitivity seems not attributable to the methods used. Labeling in glucose-responsive cells was at least 8-fold higher than that in biosynthetically inactive cells, making differences easily distinguishable in autoradiographs. Furthermore, differences in cellular silver grain density are unlikely to result from unequal cellular distribution or uptake of the free leucine tracer for the following reasons: (*i*) leucine uptake by islet cells is known to be a glucose-independent process (30); (*ii*) >85% of total cellular radioactivity was Cl_3CCOOH precipitable; and (*iii*) the selected leucine concentration (50 μM) was not rate limiting for the incorporation of label into newly formed protein (unpublished data). It is also unlikely that the observed heterogeneity in the cellular glucose responsiveness results from varying degrees of cell damage inflicted during the isolation procedure because of the following: (*i*) >95% of purified cells were, indeed, found to fulfill the classical properties of cell viability (20); (*ii*) the dose–response curves in purified beta cells were comparable to those measured in intact islets or in reaggregated and cultured beta cell preparations; and (*iii*) a similar recruitment phenomenon was seen in pancreatic beta cells examined in undissociated islet tissue. The fact that the glucose-induced

recruitment was noted in single beta cell preparations indicates that the phenomenon does not reflect a gap junction-mediated activation of unresponsive cells by neighboring activated cells. The recruitment of beta cells, on the other hand, may well be related to the differences in metabolic responsiveness that have been noted in individual beta cells exposed to different glucose concentrations (17). Therefore the variable glucose sensitivity of the redox state of individual beta cells could be responsible for the glucose-induced recruitment of the cells to biosynthetic activity. Such a mechanism may involve enzyme activation(s) by oxidoreductive signals, similar to those recently described for a yeast valyl-tRNA synthetase complex (31).

To which extent the recruitment of beta cells also occurs *in situ* and regulates the rate of insulin production *in vivo* is not yet known. It is indeed possible that the isolation of islet cells from their natural environment alters the proportion of putative "resting" cells, disturbs the periodicity in cellular activities, and/or changes the glucose sensitivity of certain beta cell subpopulations. These alterations may be responsible for the fact that 10–15% of the cells appeared biosynthetically inactive under the present *in vitro* conditions. We note that a similar proportion of cells was also metabolically unresponsive to the sugar (17). In the beta cells with a glucose-inducible protein synthesis, the sugar elicited sigmoidal dose–response curves for total protein and proinsulin biosynthesis, and this effect seemed to be accomplished via a dose-dependent recruitment of beta cells to biosynthetic activity. Nevertheless, the shape and amplitude of these curves could also be influenced by additional actions of the sugar, such as amplification of the rate of protein synthesis in recruited cells or a selectivity in its recruiting action, for example, by selecting in a dose-dependent manner cells that synthesize progressively more proinsulin than other islet proteins. Both mechanisms of action fit with the observation that glucose favors, in a dose-dependent manner, the synthesis of insulin immunoreactive material as compared with that of noninsulin proteins.

Cell recruitment has been classically associated with the neural stimulation of muscle contraction (32–35), but recent studies have extended its possible involvement to regulation in other tissues (16, 36, 37). The availability of pure and single-islet beta cells allowed us to directly demonstrate cell recruitment in endocrine cells and to measure its regulatory role in insulin production. Glucose was found to determine the degree of beta-cell recruitment to a protein synthetic activity and may well regulate the rate of insulin production *in vivo* through this mechanism. The idea that overall tissue responses can express a dose-dependent recruitment of functionally diverse cells may be of general biologic importance.

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