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Glucose-induced insulin release depends on functional cooperation between islet cells

(purified islet cell/cell coupling/intercellular communication)

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ABSTRACT **Similar to other endocrine glands, the endocrine pancreas displays a characteristic topography of its constituent cells. The functional significance of this structural organization was examined by measuring the secretory activity of the B cell in rat islet cell preparations of different composition. Glucose released 30-fold more insulin from B cells lodged within intact islets as from purified single B cells; structurally coupled B cells and single B cells isolated with A cells or incubated with glucagon responded 4- and 2-fold, respectively, more effectively to glucose than single B cells alone. Glucose homeostasis is thus dependent not only on the number and integrity of the insulin-containing B cells but also on their interactions with neighboring B and non-B cells. This study provides direct support for the concept that the microanatomy of the islet creates the anatomical basis for functional cooperation between islet cells and hence for an appropriate glucose-induced insulin release.**

The endocrine tissue in adrenal cortex, pituitary, hypothalamus, and pancreas consists of various cell types that display close contacts with homologous and heterologous cells (1–5). It is so far unknown whether this structural organization is a prerequisite for a normal endocrine response and whether it represents a possible site for endocrine disease.

We have examined the eventual role of the microanatomy of the pancreatic islet in its insulin response to glucose. The mammalian pancreas is composed of more than a thousand islets of Langerhans, each containing at least three different cell types, which are arranged according to a precise topography (6–9). Within each islet, the various endocrine cells are thought to communicate with each other, either extracellularly via their secretory products or intracellularly via cell junctions (10). The paracrine route was proposed in early reports on the insulin-stimulating effect of glucagon (11) and the inhibitory effect of another islet peptide (12), later identified as somatostatin (13, 14). An intercellular exchange through membrane channels was first suggested by the detection of gap junctions in islets (5) and later documented by electrotonic and metabolic coupling experiments (15–17). Evidence implicating both forms of intercellular communication in islet cell function and in particular in the insulin release process remains, however, largely indirect (10). To examine such eventual participation more directly, we developed a purification procedure for islet cells, which resulted in three different preparations of insulin-containing B cells: the first consisted of 25% B and 75% non-B cells, the second consisted of more than 90% single B cells, and the third consisted of 30% single and 65% structurally coupled B cells (18). Comparison of the insulin response of each of these cell suspensions with that of intact islets indicated the dependency of glucose-induced insulin release on an intercellular communication between islet cells.

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MATERIALS AND METHODS

Islets were isolated from pancreases of male Sprague–Dawley rats (250–300 g) by using the collagenase technique of Lacy and Kostianovsky (19). Groups of 1,000–2,000 islets were dissociated in calcium-free Krebs–Ringer solution containing trypsin at 10 mg/dl (Worthington) and DNase at 0.2 mg/dl (1,000 units/mg; Boehringer Mannheim, Federal Republic of Germany) (18). The islet cell purification was based on differences in sedimentation velocity; the technique of counterflow centrifugation was used to separate an A-cell-enriched fraction from single B cells and structurally coupled B cells (18). The purified fractions were suspended at 15,000 cells/ml in tissue culture medium [CMRL-1066 medium (without glutamine; GIBCO) supplemented with L-glutamine at 0.1 mg/ml, streptomycin at 0.1 mg/ml, penicillin at 0.1 mg/ml, and 10% (vol/vol) heat-inactivated fetal calf serum], distributed over 7-ml fractions into 25-cm^2 Nunclon tissue culture flasks (Nunc, Kampstrup, Denmark) and incubated at 37°C in humidified 95% air/5% CO_2; intact islets were kept under identical conditions.

After 16 hr, the cells or islets were removed from the culture flasks and filtered through 2 ml of isotonic Percoll (density, 1.045 g/ml) to remove cellular debris (18). The filtered cell pellets were suspended in 0.5 ml of CMRL-1066 medium, a 10-μl sample was taken for direct cell counting and trypan blue staining, and a 30-μl sample for pancreatic hormone assay (18); in three series of experiments, cell samples were also processed for electron microscopy (18). Finally, a volume containing 10^5 islet cells was mixed with 100 μl of preswollen Bio-Gel P2 beads (>400 mesh; Bio-Rad) and placed on top of a Bio-Gel P2 column (0.7-ml bead volume after packing at a flow rate of 1 ml/min); a similar procedure was followed in islet studies, using groups of 100 islets suspended in polyacrylamide columns. The columns were then placed in a water bath (37°C) and perifused at a constant rate of 0.8 ml/min with CMRL-1066 medium supplemented with 0.5% bovine serum albumin (fraction V, Sigma) and continuously gassed with 5% CO_2/95% air. This perifusion system was slightly modified from that originally described for pituitary cells (20) and later applied to islet cells (21).

Each perifusion experiment consisted of an initial 45-min equilibration period with 5.6 mM glucose followed by a 50-min stimulation with 20 mM glucose. The effect of 1 mM caffeine was measured during the last 25 min of glucose stimulation, first in the absence (15 min) and then in the presence (10 min) of 1 μM epinephrine. Single B cells were also tested with glucagon present throughout the perifusion (2 ng/ml, purified porcine glucagon; Novo, Denmark). The effluent was collected in 1-min fractions by using an LKB fraction collector and assayed for its insulin content (18). At the end of the perifusion, the gels were removed from the columns and examined for their pancreatic

hormone content; in at least five experiments, the islet cells were separated from the polyacrylamide beads by differential sedimentation in Krebs–Ringer medium and in isotonic Percoll (density, 1.075 g/ml), counted, and examined after trypan blue staining.

Results were calculated as ng of insulin released per min from 10^5 cells and are plotted as percentage of basal insulin levels measured at 5.6 mM glucose. The insulin response to 20 mM glucose was also expressed as the amount of hormone released above basal insulin levels; first-phase release is defined as the mean of the three peak values measured within 5 min after exposure to 20 mM glucose and the average release at 60–70 min after exposure was taken as the parameter for second-phase release. Because differences in hormone content can *per se* lead to differences in hormone release, all secretory rates were related to the corresponding cellular insulin content. Significance levels of differences compared with the single B-cell response were calculated by Student's *t* test.

RESULTS

Islet Cell Preparations. Four islet cell fractions were obtained that differed in cellular organization and composition (Table 1). Four different B-cell populations were thus recognized: intact islets, single B cells suspended with mostly glucagon- and somatostatin-containing cells, purified single B cells, and purified coupled B cells in which 40% of the cells were present in aggregates of four to eight cells and 25% as doublets or triplets; freeze-fracture replicas of the latter fraction showed the existence of junctional complexes between B cells. The trypan blue exclusion test indicated a viability of more than 90% in each fraction, and electron micrographs showed well-granulated and ultrastructurally intact cells. The perifusion procedure did not provoke significant cell loss from the columns nor was it detrimental to the cells, as shown by hormone assays, cell counts, and dye exclusion tests on samples recovered from the perifusion columns; furthermore, the percentages of single cells in perifused islet cell preparations were comparable with the before-perifusion values. No information is available on the proximity of A to B cells within the perifusion columns.

Basal Release. Under basal conditions (5.6 mM glucose), relatively constant insulin levels were measured in the perifusate collected from the 25th min on. The basal insulin release rate was therefore calculated as the mean of the values measured at 30–45 min. This parameter did not differ significantly for the three purified islet cell fractions but was significantly higher for intact islets than for purified B cells (Table 2).

Release from Purified Single B Cells. Increasing the glucose concentration from 5.6 to 20 mM provoked a biphasic insulin release from both intact islets and single B cells (Fig. 1). The response of single B cells was, however, only 1/30th of that of B cells lodged within islets; a similar difference was observed when the released hormone was expressed as the percentage of the corresponding insulin content (Table 2). Addition of caffeine increased the insulin response to glucose 3-fold ($P < 0.01$), which contrasts with the 50% increase in single B-cell preparations.

Effect of Glucagon on B-Cell Function. Glucose-induced insulin release from single B cells isolated with mostly A cells followed the same biphasic pattern as single B cells alone but reached significantly higher values, both in terms of percentage of basal values (Fig. 2) and with respect to the insulin content (Table 2); the effluent of columns filled with A-cell-rich fractions contained glucagon at 0.72 ± 0.08 ng/ml. The stimulatory effect of 20 mM glucose on single B cells was also enhanced by the inclusion of glucagon (2 ng/ml) in the perifusion media (Table 2). However, the presence of A cells or of glucagon did not increase the potentiating effect of caffeine on single B cells.

Effect of Cell Coupling on B-Cell Function. The role of cell coupling in hormone release was studied by comparing the secretory responses of two purified B-cell preparations that differed in their percentage of structurally coupled cells. At the high-glucose concentration, coupled B-cell suspensions (with more than 65% of their cells incorporated in aggregates of 2–10 cells) released 4-fold more insulin than single B-cell fractions (with less than 30% of the cells aggregated in clumps of up to 4 cells) (Fig. 2 and Table 2). The higher secretory responsiveness of coupled B cells became even more pronounced in the presence of caffeine (Fig. 2).

In contrast to the various stimulatory effects of glucose and caffeine on the four B-cell preparations, the addition of epinephrine inhibited glucose-induced insulin release promptly and completely in each islet cell fraction (Figs. 1 and 2).

DISCUSSION

Glucose homeostasis is greatly dependent on the rapidity and intensity of the B-cell secretory response to glucose. Perifusion techniques were therefore developed to examine the dynamics and regulation of insulin release from the entire pancreas (22), from fresh (23) or cultured (24) islets, and from islet cell suspensions (21, 25) or monolayers (26). As we were interested in comparing the secretory capability of single and coupled B cells, we selected the cell column method of Lowry (20) and perifused intact islets and purified islet cells while they were suspended with an excess of small polyacrylamide beads (diameter, <40 μm). The usefulness of this procedure is indicated by the prompt and intense biphasic insulin release from glucose-stimulated islets and by its ability to maintain the cellular composition and viability of purified islet cell fractions.

Purified single B cells released 1/30th as much insulin in response to 20 mM glucose than B cells lodged within intact islets. These lower values were not caused by differences in basal release nor by a cellular degranulation but rather correspond to a decreased responsiveness to glucose. Several arguments make it unlikely that this poor secretory capability results from some damage during the purification procedure (for example, by exposure to trypsin): (*i*) the cells appeared morphologically intact as judged from electron micrographs and dye exclusion tests, whereas their sensitivity to a low concentration of glucagon and of epinephrine fulfills a generally accepted cri-

Table 1. Cellular and hormonal compositions of the four islet cell preparations

Preparation	Cell type, % total				Hormone content, μg/10^3 islets or 10^6 cells		
	Single	A	B	D	Insulin	Glucagon	Somatostatin
Islets	?	20–25	60–70	5–10	65.5 ± 7.4	1.8 ± 0.2	0.17 ± 0.02
A-cell-rich fraction	>80	60–65	20–25	5–10	11.2 ± 2.2	6.5 ± 0.7	0.22 ± 0.03
Single B cells	>70	<8	>90	<5	85.3 ± 7.9	0.4 ± 0.05	0.11 ± 0.02
Coupled B cells	<35	<5	>90	5–8	103.1 ± 9.8	0.2 ± 0.03	0.21 ± 0.03

Hormone contents are expressed as mean ± SEM ($n = 8$).

Table 2. Basal and glucose-induced insulin release in the four B-cell preparations

Preparation	*n*	Basal, (pg/min)/ng	Glucose (20 mM)-induced, (pg above basal/min)/ng	
			First phase	Second phase
Islets	10	0.17 ± 0.05*	2.16 ± 1.00†	1.46 ± 0.56†
A-cell-rich fraction	8	0.11 ± 0.05	0.19 ± 0.07†	0.18 ± 0.06*
Single B cells	9	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.02
Coupled B cells	8	0.04 ± 0.02	0.22 ± 0.07†	0.21 ± 0.06‡
Single B cells/ glucagon (2 ng/ml)	3	0.07 ± 0.02	0.12 ± 0.03*	0.11 ± 0.03*

Results are expressed per ng of insulin content and are mean ± SD of *n* determinations. Significance of differences compared with the single B-cell response was calculated by Student's *t* test.
*$P < 0.05$.
†$P < 0.01$.
‡$P < 0.02$.

terion of membrane integrity and (*ii*) a prolonged culture period—which is known to restore trypsin-damaged cell membranes—did not improve glucose-induced insulin release from single B-cells (unpublished observations). It remains, however, difficult to absolutely exclude the possibility that coupled cells have been less affected by trypsin than single cells and were therefore better responders to glucose. We are nevertheless more attracted by the concept that the response of the B-cell to glucose is highly dependent on certain insular factors that are lost during the purification of single B cells. The results obtained with coupled B cells and with B cells isolated with A cells support this view.

Single B cells responded better to glucose when they were isolated and hence cultured and perifused with A cells. This effect was not associated with an increase in cell coupling and is rather the result of a paracrine action of glucagon: the glucagon concentrations measured in the effluent (0.6–0.9 ng/ml) indeed underestimate the actual hormone levels surrounding the B cells and are thus comparable with those that stimulate glucose-induced insulin release from purified B cells (2 ng/ml). These results are compatible with the view that the peripheral rim of A cells in the rat islet (8) plays a critical role in glucose regulation of insulin release and this through a paracrine interaction with the more centrally located B cells.

A structural coupling between B cells was associated with an increased responsiveness to glucose and to caffeine. In view of earlier reports on electrotonic and metabolic coupling between islet cells (15–17), it is tempting to attribute this phenomenon to a junctional exchange of intracellular regulators that synchronize and optimize the secretory function of the B cell. It is also conceivable that coupled cells display a better intracellular organization that could facilitate hormone transport and release (27). Single and coupled cells may also differ in their cAMP or calcium metabolism, since caffeine markedly potentiated glucose-induced insulin release from coupled B cells while it exerted only a minor effect on single B cells.

The present study indicates that single B cells are poor responders to glucose and that this characteristic is caused, at least in part, by their single occurrence and by their dependence on extracellular glucagon. The capability of intact islets to counteract a glucose challenge by a rapid and appropriate insulin release may thus require both some degree of coupling between B cells and optimal glucagon levels in the vicinity of the B cells.

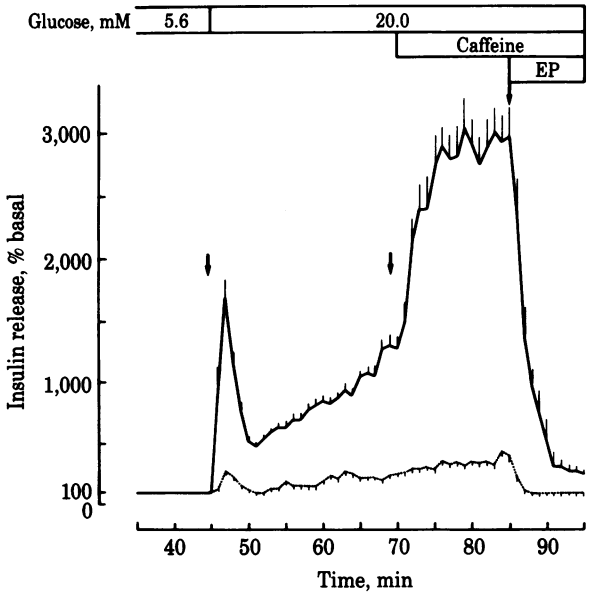

FIG. 1. Insulin release from intact islets (solid line, $n = 10$) and from single B cells (dotted line, $n = 9$). Results are expressed as percentage of the hormone release at 5.6 mM glucose (basal) and plotted as mean ± SEM. A statistically significant difference was measured between responses ($P < 0.001$). Caffeine was used at 1 mM and epinephrine (EP) was used at 1 μM.

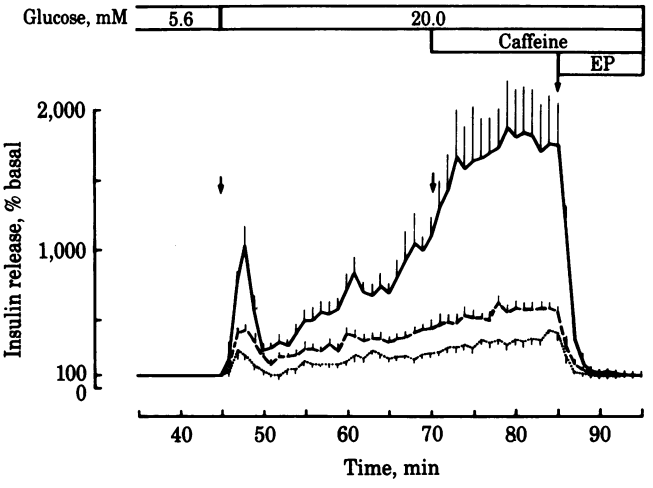

FIG. 2. Comparison of insulin release from structurally coupled B cells (solid line, $n = 8$) and from single B cells isolated and incubated with A cells (broken line, $n = 8$) with that from single B cells (dotted line, $n = 9$). Results are expressed as in Fig. 1. The significance level of the difference from the single-B-cell response was $P < 0.001$ for first-phase insulin release (both conditions), $P < 0.02$ for the second phase of coupled B cells, and $P < 0.05$ for the second phase of the A-cell-enriched fraction. Caffeine was used at 1 mM and epinephrine (EP) was used at 1 μM.

It is concluded that the main event in glucose homeostasis—namely, glucose-induced insulin release—is not only dependent on the number and integrity of the individual B cells but also on the microanatomy and functional organization of the islet. Physiological, pharmacological, and pathological alterations in insulin release are thus best examined in purified islet cell preparations, to distinguish direct effects on the B cell from those on its intercellular communication. Further experiments are now required to analyze the functional cooperation within the endocrine pancreas at a subcellular level and to assess its occurrence in other (neuro)endocrine tissues.

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