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MIN6 β-cell–β-cell interactions influence insulin secretory responses to nutrients and non-nutrients

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Abstract

Insulin-secreting MIN6 cells show greatly enhanced secretory responsiveness to nutrients when grown as islet-like structures (pseudoislets). Since β-cells use different mechanisms to respond to nutrient and non-nutrient stimuli, we have now investigated the role of homotypic β-cell interactions in secretory responses to pharmacological or receptor-operated non-nutrient stimuli in MIN6 pseudoislets. In addition to an enhanced secretory responsiveness to glucose, insulin secretion from MIN6 pseudoislets was also enhanced by non-nutrients, including carbachol, tolbutamide, PMA, and forskolin. The improved secretory responsiveness was dependent on the cells being configured as pseudoislets and was lost on dispersal of the pseudoislets into single cells and regained on the re-formation of pseudoislet structures. These observations emphasise the importance of islet anatomy on secretory responsiveness, and demonstrate that homotypic β-cell interactions play an important role in generating physiologically appropriate insulin secretory responses to both nutrient and non-nutrient stimuli.

Keywords: Pancreatic β-cell; Islet of Langerhans; Insulin secretion; Nutrient; Non-nutrient

The generation of appropriate patterns of insulin secretion from pancreatic β-cells is dependent upon the anatomical configuration of β-cells within the islets of Langerhans. It has been known for many years that disrupting the islet architecture results in reduced glucose-induced insulin release from β-cells, and that reaggregation of the islet cells can partially restore nutrient-induced insulin secretory responses [1–3].

Several mechanisms are likely to contribute to the intra-islet integrative process, making the islet of Langerhans a complex and heterogeneous experimental model for studies of cell–cell interactions. We have, therefore, developed an in vitro experimental model in which to selectively investigate the functional consequences of homotypic interactions between β-cells by configuring insulin-secreting MIN6 cells, which normally grow as adherent monolayers, into islet-like structures (which we have previously called pseudoislets, see [4,5]). MIN6 pseudoislets showed greatly enhanced secretory responsiveness to nutrients when compared to equivalent cells configured as monolayers, suggesting that interactions between β-cells are alone sufficient to maintain normal responses to nutrients, independently of interactions with other types of islet cells [4,5].

While a rise in the extracellular glucose concentration is required to initiate an insulin secretory response, the magnitude of the response is regulated by a number of neurotransmitters and hormones that act through conventional cell-surface receptors. The cellular mechanisms through which pancreatic β-cells recognise external stimuli are becoming increasingly well understood, and it is clear that β-cells use different mechanisms and intracellular signalling pathways to respond to nutrients and to non-nutrients [6,7]. In the present study, we have investigated whether

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homotypic β-cell interactions influence secretory responses to pharmacological and receptor-operated non-nutrient stimuli.

Materials and methods

Materials

MIN6 cells were obtained from Dr. Y. Oka and Professor J.-I. Miyazaki (University of Tokyo, Tokyo, Japan). β TC3 cells were a kind gift from Dr S. Efrat (then at Cold Spring Harbor Laboratory, New York 11724). All tissue culture reagents, forskolin, and phorbol myristate acetate (PMA) were purchased from Sigma (Poole, Dorset, U.K.).

Methods

Maintenance of cells and pseudoislets. MIN6 β-cells and pseudoislets, passage 43–53 were cultured as described in [8]. Pseudoislets were used for experiments 6–8 days after subculturing, and compared in the same experiments to equivalent passage monolayer cells. For some experiments dispersed pseudoislet cells were reaggregated by a further culture period of 6–8 days on gelatin.

Insulin secretion. Monolayer cells were harvested from the tissue culture plastic substrate using a non-tryptic method (EDTA 0.02% w/v solution in PBS), and pseudoislets were harvested by aspiration. Pseudoislets were disaggregated by incubation in the presence of EDTA (0.02% w/v, 5 min, 37 °C) followed by gentle trituration until single cell suspensions were achieved. Dispersed cells and pseudoislets were pelleted by centrifugation (1000*g* 5 min) and resuspended in a bicarbonate-buffered physiological salt solution [9]. The rates and patterns of insulin secretion from cells and pseudoislets were studied using a multi-channelled temperature-controlled (37 °C) perifusion system, as described previously [5,10] with similar numbers of cells being loaded as pseudoislets or as single cell suspensions.

Data analysis. Data are expressed as means ± SE, where n = number of separate perifusion channels in one experiment representative of three or more separate experiments. Differences between treatments were assessed using one-way analysis of variance, unpaired or paired Student's t test, Dunnett's t test, and Bonferroni's multiple comparisons test, as appropriate. Differences between treatments were considered significant when $P < 0.05$.

Results

Insulin secretion in response to depolarising stimuli

MIN6 cells grown as monolayers or as pseudoislets had a similar insulin content (monolayer: 13.3 ± 1.4 ng insulin/μg protein; pseudoislet: 12.4 ± 0.6 ng insulin/μg protein, mean ± SEM, $n = 3$, $P > 0.2$) and basal rate of insulin secretion (monolayers: 0.42 ± 0.06 ng insulin/μg protein/h; pseudoislets 0.35 ± 0.04 ng insulin/μg protein/h, means ± SEM, $P > 0.2$, $n = 8$), confirming previous reports that aggregation did not affect insulin content nor the basal rate of insulin secretion [4]. The rate of stimulated secretion in perifusion experiments was therefore expressed as a percentage of the basal secretion (average secretion over the first 10 min) to compensate for any differences in tissue loading between perifusion chambers. The enhanced secretory responsiveness to glucose of MIN6 cells configured as pseudoislets is demonstrated in Fig. 1A. Increasing the glucose concentration from 2 to 20 mM caused a rapid, biphasic, and maintained increase in insulin secretion from pseudoislets, with an initial peak followed by a sustained plateau. Suspensions of equivalent passage monolayer MIN6 cells also responded to 20 mM glucose (Fig. 1A), but the amplitude of the response was much reduced

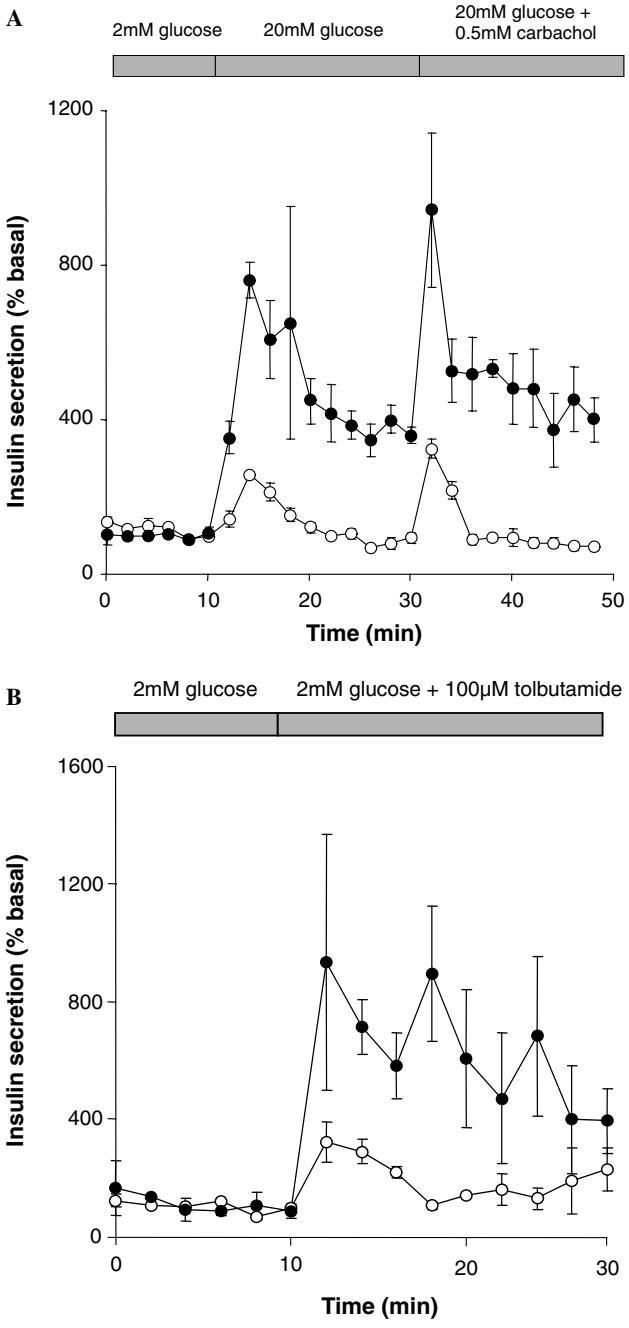

Fig. 1. Effects of a receptor-operated agonist on insulin secretion from MIN6 monolayers and pseudoislets. (A) MIN6 monolayer cells (○) and pseudoislets (●) were perifused with buffer containing 2 mM glucose, 20 mM glucose, or 20 mM glucose supplemented with the cholinergic agonist carbachol (0.5 mM), as shown by the horizontal bars. Secretion is expressed as a percentage of basal secretion (means ± SE, $n = 3$). (B) MIN6 monolayer cells (○) and pseudoislets (●) were perifused with buffer containing 2 mM glucose or 2 mM glucose and tolbutamide (100 μM), as shown by the horizontal bars. Secretion is expressed as a percentage of basal secretion (means ± SE $n = 3$).

compared to that of pseudoislets and secretion was not maintained. The enhanced secretory function of pseudoislets over equivalent monolayer cells also extended to receptor-operated stimuli. Thus, the acetylcholine analogue, carbachol (CCh) caused a rapid, biphasic, and maintained potentiation of glucose-induced insulin secretion from pseudoislets, as shown in Fig. 1A. In contrast, monolayer cells showed a smaller secretory response to glucose which returned to basal values within 10 min, and CCh produced only a transient monophasic increase in the glucose-induced secretory response. These observations were confirmed when perifused MIN6 cells and pseudoislets were exposed to tolbutamide, as shown in Fig. 1B. Thus, in the presence of a sub-stimulatory concentration of glucose (2 mM) tolbutamide (100 μM) elicited a rapid and maintained secretory response from pseudoislets, compared to a much lesser and transient response from suspensions of equivalent passage monolayer MIN6 cells.

Glucose-induced insulin secretion can be potentiated by pharmacological activation of diacylglycerol-dependent protein kinase C (PKC) or cyclic AMP-dependent protein kinase A (PKA), using PMA or forskolin, respectively. Both of these agents enhanced glucose-induced insulin secretion from MIN6 cells, and the enhancement was much greater in pseudoislets compared to equivalent monolayer cells, whether in response to the activation of PKC (Fig. 2A) or of PKA (Fig. 2B).

Fig. 2 also shows that dispersed pseudoislet cell populations showed greatly reduced responses to glucose and kinase activators. There were no statistically significant differences in insulin secretion from monolayer cells or dispersed pseudoislet cells in response to glucose,

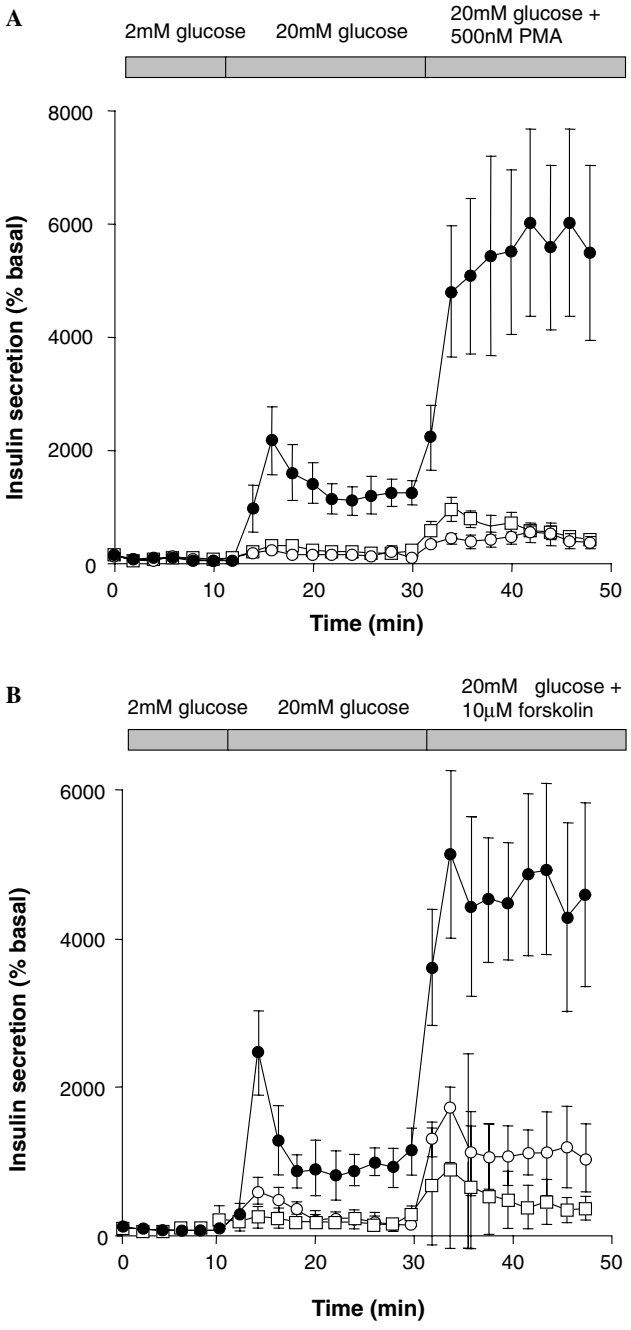

Fig. 2. Effects of protein kinase activators on insulin secretion from MIN6 monolayers, pseudoislets, and dispersed pseudoislets. (A) MIN6 monolayer cells (○), pseudoislets (●), and dispersed pseudoislets (□) were perifused with buffer containing 2 mM, 20 mM glucose or 20 mM glucose supplemented with the PKC activator PMA (500 nM), as shown by the horizontal bars. Secretion is expressed as a percentage of basal secretion (means ± SE, $n = 3$). (B) MIN6 monolayer cells (○), pseudoislets (●), and dispersed pseudoislets (□) were perfused with buffer containing 2 mM glucose, 20 mM glucose or 20 mM glucose supplemented with the adenylate cyclase activator forskolin (10 μM) and the phosphodiesterase inhibitor IBMX (100 μM), as shown by the horizontal bars. Secretion is expressed as a percentage of basal secretion (means ± SE, $n = 3$).

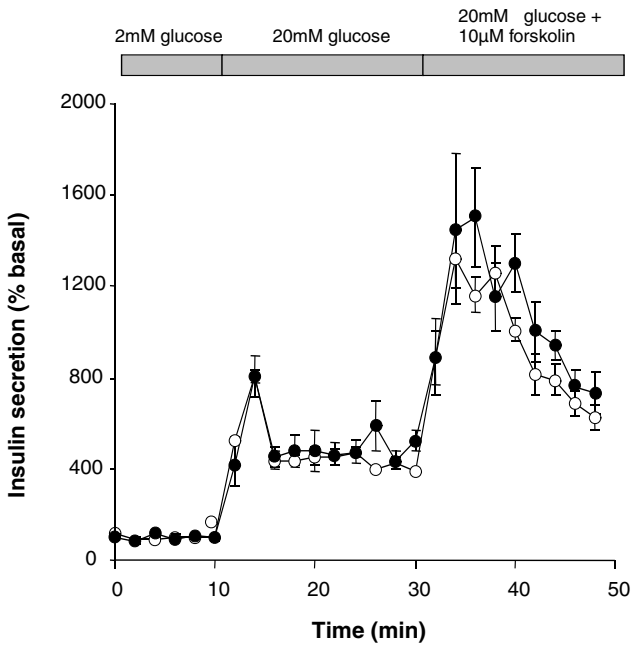

Fig. 3. Reaggregation restores secretory responses of dispersed pseudoislet cells. MIN6 pseudoislets were generated from monolayer cells (●) or from dispersed pseudoislet cells (○) and perifused with buffers containing 2 mM glucose, 20 mM glucose or 20 mM glucose supplemented with forskolin (10 μM) and IBMX (100 μM), as shown by the horizontal bars. Secretion is expressed as a percentage of basal secretion (means ± SE, $n = 3$).

PMA or forskolin ($P > 0.2$) (Fig. 2A and B, respectively).

In a subsequent series of experiments, dispersed MIN6 pseudoislet cells were reaggregated into pseudoislets by re-seeding onto gelatin-coated tissue culture plastic and culturing for 6–8 days, as described under Methods, and the secretory responses of these reaggregated pseudoislets were compared to pseudoislets generated directly from equivalent passage monolayer cells. Fig. 3 demonstrates that reaggregation restored secretory responses of dispersed pseudoislet cells. Thus, reaggregated pseudoislets showed identical secretory responses ($P > 0.2$) to those of pseudoislets derived from monolayer MIN6 cells when exposed to either glucose (20 mM) alone or to forskolin (10 μM) in the presence of glucose.

Discussion

It is well known that the secretory function of β-cells is dependent upon their anatomical configuration within the islets of Langerhans [2,3,11], and that dispersed islet cells can reform islet-like structures in vitro [12]. Our current results demonstrate that the enhanced secretory function induced by interactions between β-cells in islet-like clusters is not confined to nutrient stimuli [4], since MIN6 cells grown as pseudoislets also showed greatly enhanced responses to a receptor agonist, to a sulphonylurea, and to kinase activators. However, the current study also demonstrates that enhancement of insulin secretory responses caused by cell aggregation was dependent on the continued anatomical configuration of the cells as aggregates in MIN6 pseudoislets since it was immediately lost on the disaggregation of the pseudoislet structure, and could be regained by reaggregation of the dispersed insulin-secreting cells into pseudoislets. These observations support the concept of improved insulin secretion being a consequence of short-term cell–cell interactions within the islets, rather than being caused by phenotypical changes in the individual cells that comprise the islet by the contact-induced changes in gene expression that have been reported in other experimental models [13–16]. Furthermore, we have previously reported [4], and here confirm, that there is no significant difference in the insulin content of MIN6 cells configured as monolayers or pseudoislets, consistent with our previous report that pseudoislet formation did not affect the proliferative capacity or differentiated state of the MIN6 cells [8].

Elevations in intracellular Ca^{2+} ($[Ca^{2+}]_i$) regulate insulin secretion by linking nutrient metabolism to the initiation of exocytosis, and communication through gap junctions has been implicated in the synchronous changes in $[Ca^{2+}]_i$ induced in islets and MIN6 cells in response to nutrient secretagogues [17–22]. However, the $[Ca^{2+}]_i$ responses of isolated primary β-cells [23] and MIN6 cells [4] are heterogeneous, and β-cell heterogeneity has been demonstrated at a number of different levels in the signalling pathway leading to insulin secretion [24–29]. We have previously reported that configuring MIN6 cells as pseudoislets reduced the heterogeneity of their $[Ca^{2+}]_i$ responses to glucose [4]. This effect was immediately reversed by disaggregation of the pseudoislets into single cell suspensions, in accordance with the effects on glucose-induced insulin secretion in the present study. These observations are consistent with a heterogeneous β-cell population in which only a sub-population of cells respond to stimulatory concentrations of glucose by secreting insulin. When configured as pseudoislets, the responsive cells recruit adjacent non-responding cells into synchronised $[Ca^{2+}]_i$ and insulin secretory responses, but the loss of cell–cell apposition in cell suspensions or monolayers prevents this integration [4,27,30]. This model can explain our current observations of enhanced insulin secretion from MIN6 cells in pseudoislets in response to a cholinergic receptor agonist, and to the activation of PKC or PKA. Thus, it is generally accepted that nutrient stimuli initiate insulin secretion from β-cells while receptor-operated agonists or pharmacological activators of protein kinases enhance nutrient-induced insulin secretion but have little or no effect on secretion in the absence of stimulatory concentrations of glucose [7,31]. The increased secretory responses of MIN6 pseudoislets to non-nutrients most likely reflect the increased proportion of cells that are activated by glucose when in pseudoislets, enabling the enhancement of glucose-initiated responses of these cells by non-nutrients. Our experiments also demonstrated that MIN6 cells configured as pseudoislets showed enhanced secretory responses to direct depolarisation by K_{ATP} channel closure using tolbutamide, which bypasses the metabolic pathways of glucose recognition. This observation suggests the existence of a mechanism through which homotypic β-cell interactions enhance insulin secretion independent of K_{ATP}-dependent β-cell depolarisation. It is now well established that β-cells recognise and respond to nutrients through both K_{ATP}-dependent and K_{ATP}-independent pathways, although the relative physiological importance of each pathway is still uncertain [32]. Our results are consistent with the enhanced secretory responses of β-cells in islet-like structures being due, at least in part, to recruitment of non-responsive cells through K_{ATP}-independent signalling pathways.

In conclusion, our results suggest that the formation of islet-like structures is an inherent property of β-cells. Homotypic β-cell communication in these structures results in enhanced insulin secretory responses to nutrients and non-nutrient stimuli that are completely, and reversibly, dependent on the anatomical configuration of the β-cells within the islet-like structures. There are numerous potential mechanisms through which adjacent cells within islets can communicate [33–46], and functional integration of islet responses may involve several coordinating mechanisms. The present study suggests that at least some of these are specific to beta cells, and offers a model in which to determine the relative importance of the different forms of β cell interactions.

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