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Islet α-cells do not influence insulin secretion from β-cells through cell–cell contact

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Abstract Interactions between the endocrine cells in islets of Langerhans influence their secretory function, and disruption of islet structure results in impaired insulin secretory responses to both nutrient and non-nutrient stimuli. We have previously demonstrated that insulin-secreting MIN6 cells show enhanced secretory responses when grown as islet-like structures (pseudoislets) suggesting that homotypic cell–cell interactions between β-cells are important for normal function. We have now extended this experimental model to study the role of heterotypic interactions between insulin-expressing and glucagon-expressing cells by measuring the organization and secretory function of pseudoislets formed from MIN6 and αTC1 cells. The direct α-cell to β-cell contact in the heterogenous MIN6/αTC1 pseudoislets was sufficient to enable the formation of anatomically correct islet-like structures, with a central core of MIN6 cells surrounded by a periphery of αTC1 cells. However, the presence of αTC1 cells had no detectable effect on insulin secretory responses to nutrient or non-nutrient stimuli. In contrast, exogenous glucagon enhanced insulin secretion, in accordance with a paracrine role for α-cell-derived glucagon in the regulation of insulin secretion rather than direct, contact-mediated effects of α-cells on neighbouring β-cells.

Keywords Pancreatic β-cell · α-Cell · Islet of Langerhans · Insulin secretion

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Introduction

The endocrine islets of Langerhans possess a highly organised three-dimensional architecture, the integrity of which is required for optimal insulin secretory responses by the islet β-cells [1–3]. A number of mechanisms have been invoked to account for the enhanced performance of β-cells when configured as part of an islet-like structure. Communication between islet cells can occur by direct cell–cell contact through cell adhesion molecules [4–7], by the exchange of small molecules and/or ions via gap junctions [8–10] and by paracrine interactions through various secreted products [11–16], and it seems likely that normal functioning of the islet is dependent, to some extent, on each of these forms of inter-cellular communication [17].

The existence within a primary islet of at least four separate endocrine cell types, in addition to a variety of non-endocrine cell types, makes it a complex and heterogeneous experimental model for studies of cell–cell interactions. We have developed in vitro experimental models to investigate interactions between islet endocrine cells by studying the functional consequences of configuring insulin-secreting MIN6 cells or glucagon-secreting αTC1 cells, which normally grow as adherent monolayers, into islet-like structures, which we have previously referred as *pseudoislets* [18–21]. These studies have demonstrated that direct homotypic cell–cell interactions between insulin-secreting β-cells are sufficient to greatly enhance insulin secretory responses to nutrient [19, 20] and non-nutrient [18, 21] stimuli. In contrast, glucagon secretion is not dependent on homotypic interactions between α-cells [18]. These observations are consistent with the anatomical organisation of rodent islets, in which β-cells in the core of the islet are in contact with many other β-cells, while α-cells in the islet mantle have much less potential for contact with other α-cells.

In addition to homotypic cell interactions in islets there are also evidences for heterotypic interactions between different islet endocrine cells, in particular between α- and β-cells. Thus, gap junctional coupling has been reported between α- and β-cells [10], and numerous studies suggest that α-cells may regulate insulin secretion by paracrine effects of glucagon on the β-cell [12, 22, 23]. However, previous studies using primary islets have been unable to discriminate between influences mediated by direct cell–cell contact between α- and β-cells (e.g. gap junctions, cell adhesion molecules) and paracrine effects secondary to intra-islet hormone release. In the present study we have extended our experimental model to study the effects of heterotypic interactions between α- and β-cells on insulin secretion, and to determine the likely mechanism of these effects.

Materials and methods

Materials

MIN6 cells were obtained from Dr. Y. Oka and Professor J.-I. Miyazaki (University of Tokyo, Japan). The αTC1 cells were obtained from Dr. Lamin Marenah (University of Ulster, Northern Ireland). Tissue culture reagents, glucagon, mouse monoclonal anti-glucagon antibody, phorbol myristate acetate (PMA) and general chemicals were purchased from Sigma (Poole, Dorset, U.K.). Guinea pig anti-insulin antibody was from ICN Biomedical (California, USA). [^{125}I]-glucagon for radioimmunoassay (RIA) and ^{125}I for insulin iodination were obtained from Amersham Biosciences (Amersham, UK).

Methods

Maintenance of cells and pseudoislets

MIN6 cells and αTC1 cells were maintained at 37°C/5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin and 2 mmol/l glutamine. The medium was changed every 3 days and the monolayers were passaged and used for experiments when 70% confluent. Pseudoislets were generated by culturing MIN6 cells or mixed populations of MIN6 and αTC1 cells (75%/25% ratio) for 6–8 days on tissue culture flasks that had been pre-coated with 2% gelatin, as described previously [18, 24].

Immunocytochemistry

Pseudoislets were fixed in 4% paraformaldehyde, incubated overnight in 30% sucrose, embedded in optimum cutting temperature (O.C.T.) compound and frozen in liquid nitrogen. Frozen sections were cut at 7 μm and stained with haematoxylin and eosin (H&E) stain using Gill's haematoxylin solution for histological examination. Insulin and glucagon immunoreactivities were localised by immunostaining with anti-glucagon (1:500) and anti-insulin (1:50) antibodies, using the Dako Envision Double Stain Kit, according to the manufacturer's instructions. Micrographs of immunostained pseudoislet sections were analysed by assessing the location of all the glucagon-immunoreactive cells in each section. Individual αTC1 cells were defined as *peripheral* α if they were located on the outer edge of the pseudoislet, as *outer* α if they were within 1–3 cells of the nearest outer edge and as *core* α if they were located more than 3 cells from the nearest outer edge. Examples of each type of location are shown in Fig. 1A.

Hormone content and secretion

Monolayer MIN6 and αTC1 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. Cells were pelleted by centrifugation (1000 g, 5 min), and hormone content was extracted in acidified ethanol [19] and measured by RIA for insulin [25] or glucagon [26]. Insulin secretion from MIN6 or MIN6/αTC1 pseudoislets was assessed using a temperature-controlled (37°C), multi-channel perifusion system [18, 20]. Pseudoislets were loaded on to 1 μm nylon filters in Swinnex filter holders and perifused (0.5 ml/min) with a bicarbonate-buffered physiological salt solution [27] supplemented with 2 mM glucose, 2 mM $CaCl_2$, and 0.5 mg/ml bovine serum albumin. Fractions were collected every two minutes, and insulin content was assessed by RIA.

Data analysis

Data are expressed as mean ± SEM. Differences between treatments were assessed using one-way analysis of variance, and unpaired or paired Student's *t*-test, as appropriate. Differences between treatments were considered significant when $p < 0.05$.

Results

Pseudoislet formation and structure

Maintaining mixed populations of MIN6 (75%) and αTC1 (25%) cells in culture for 6–8 days on gelatin-coated tissue culture plastic led to the formation of three dimensional islet-like clusters which were similar in appearance to those

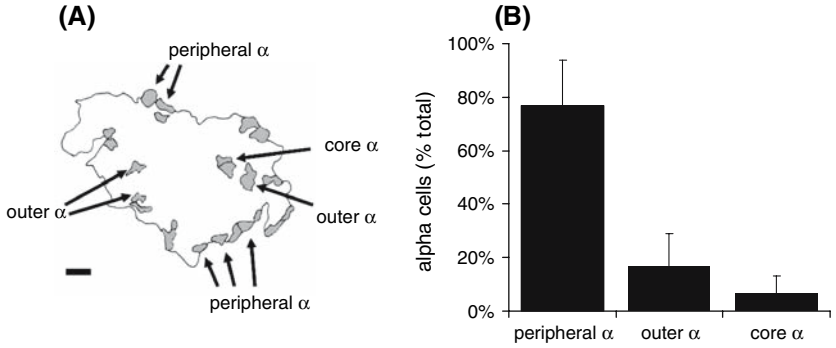


Fig. 1 Anatomical organization of heterotypic MIN6/αTC1 pseudoislets (**A**) The schematic diagram shows a tracing of a typical section of a MIN6/αTC1 pseudoislet after identification of the cells by immunostaining for insulin and glucagon. For clarity only the glucagon immunoreactive αTC1 cells are shown (shaded). Individual αTC1 cells were defined as *peripheral* α if they were located on the outer edge of the pseudoislet, as *outer* α if they were within 1–3 cells of the nearest outer edge and as *core* α if they were located more than 3 cells from the nearest outer edge, as shown on the schematic. Scale bar = 10 μm. (**B**) Most of the αTC1 cells were located in the peripheral and outer regions of the MIN6/αTC1 pseudoislet, as defined in (**A**), with the MIN6 cells comprising the majority of the core of the pseudoislet structure. Bars show means + SEM, $n = 10$.

reported previously for homogenous populations of MIN6 cells [19, 21] and αTC1 cells [19]. Immunocytochemical analysis demonstrated that these structures were not random clusters of both cell types, but that the cells spontaneously segregated into organised structures, with the majority of αTC1-cells being located in the periphery of the pseudoislet, whilst the MIN6 cells formed the core of the structure, as shown in Fig. 1A. Figure 1B shows the location of the αTC1 cells within the islet-like structures as assessed by analysis of immunostained sections. Thus, the majority of αTC1-cells were located on the outer edge of the structure, or within a few cells of the outer edge, in accordance with the segregation of endocrine cells in primary rodent islets [28].

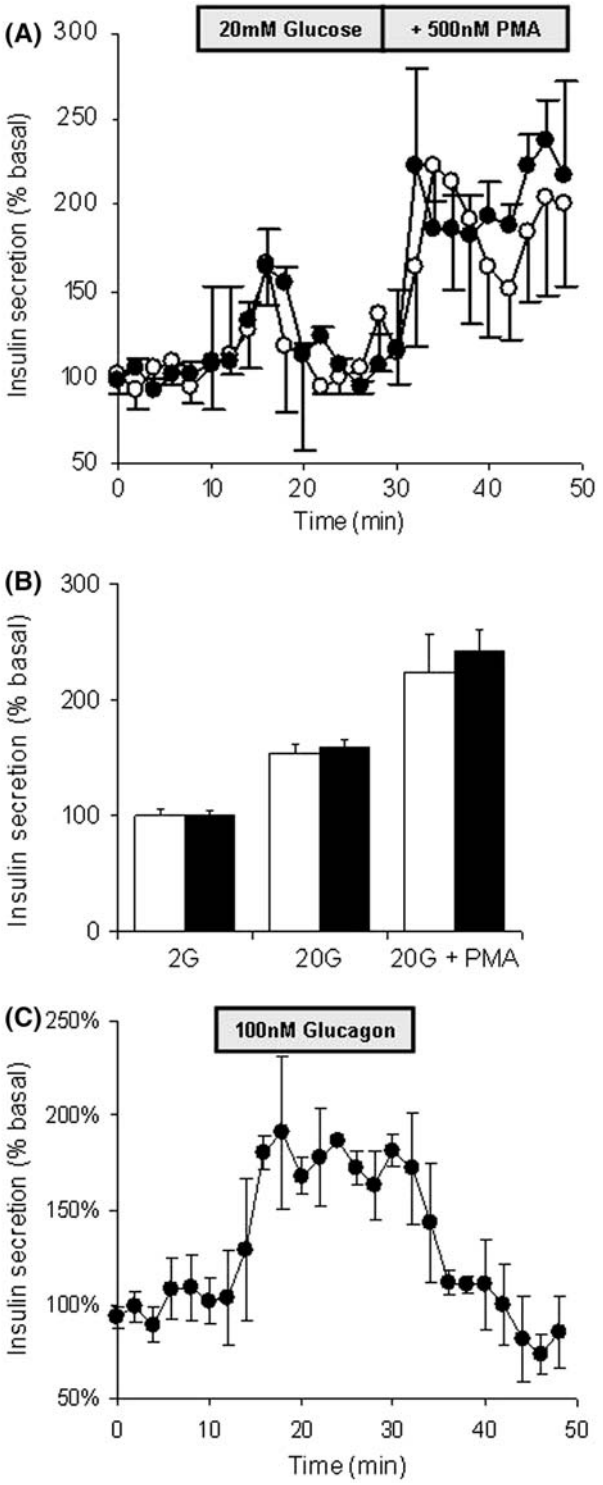
Hormone content and secretion

The glucagon content of αTC1 cells was not influenced by the anatomical configuration of the cells as monolayers or pseudoislets (monolayer αTC1 = 106 ± 8 pg glucagon/μg protein; αTC-1 pseudoislet, 110 ± 18, $n = 4$, $p > 0.2$) and insulin was undetectable in both populations (<0.8 fg/cell). This level of glucagon expression equates to 23 ± 1 fg glucagon/αTC1 cell, which is less than 1% of the glucagon content of a primary rodent α-cell [29]. In contrast, the insulin content of MIN6 cells is considerably higher, having been reported previously as being approximately 10% of a primary rodent β-cell [30], although this declines with passage [31, 32].

The rate and pattern of insulin secretory responses of homogenous MIN6 pseudoislets and heterogenous MIN6/αTC1 pseudoislets are shown in Fig. 2A. Increasing the glucose concentration from 2 mM to 20 mM glucose caused a rapid increase in insulin secretion and this glucose-induced response was further enhanced by the presence of the protein kinase C activator PMA (500 nM). Figure 2B shows that there were no detectable differences ($p > 0.2$) between the two populations of pseudoislets in the overall magnitude of the secretory responses, nor in the peak responses to glucose or PMA. Figure 2C shows the effect of exogenous glucagon on insulin secretion from homogenous MIN6 pseudoislets. In the presence of a substimulatory concentration of glucose (2 mM) glucagon (100 nM) caused a significant ($p < 0.01$), sustained and reversible increase in insulin secretion.

Discussion

Insulin-secreting β-cells and glucagon-secreting α-cells comprise the majority of endocrine cells within the islet of Langerhans, contributing approximately 70–80% and 20–30% of the total cell number, respectively [33]. Previous work has demonstrated that α- and β-cells dispersed from primary rodent islets have the ability to re-aggregate into organised, islet-like structures with the α-cells forming a mantle around a largely β-cell core [33]. These observations suggest that the islet endocrine cells possess the information required for anatomically-correct islet assembly, which is thought to be through the differential expression of cell adhesion molecules such as E-Cadherin (ECAD; [5, 19, 34]) and Neural Cell Adhesion Molecule (NCAM; [4, 35]). The present results demonstrate that the transformed endocrine cell lines being used in our studies retain the ability to self-organise into islet-like structures, in accordance with our previous reports of the differential expression of ECAD and NCAM in these two cell lines [18, 19, 24]. Thus, when mixed in a



◀ **Fig. 2** Insulin secretion from homotypic MIN6 and heterotypic MIN6/αTC1 pseudoislets. (**A**) Perifusion experiments using homogenous MIN6 pseudoislets (•) and heterogenous MIN6/αTC1 pseudoislets (○) demonstrated that both populations of pseudoislets showed similar patterns of insulin secretion in response to elevated glucose (20 mM, bar) and to a protein kinase C activator (500 nM PMA, bar). Points are means ± SEM (n = 4) with one set of error bars for each treatment omitted for clarity. (**B**) Increasing the glucose concentration from a basal of 2 mM (2G) to 20 mM (20G) caused a significant ($p < 0.01$) increase in insulin secretion from pseudoislets composed entirely of MIN6 cells (open bars) or of a mixture of 75% MIN6 cell and 25% αTC1 cells (solid bars), and this glucose-induced response was further enhanced ($p < 0.01$) in the presence of 500 nM PMA (20G + PMA). There were no significant differences ($p > 0.2$) between the secretory responses of MIN6 pseudoislets and MIN6/αTC1 pseudoislets under any of the conditions tested. Bars show means + SEM, n = 4. (**C**) Exposure to exogenous glucagon (100 nM, bar) caused a reversible, approximately two-fold increase in the basal rate of insulin secretion from MIN6 pseudoislets in the presence of 2 mM glucose. Points show means ± SEM, n = 4

physiologically-appropriate ratio of 75%/25% MIN6/αTC1 cells the cells segregated in resultant heterotypic pseudoislets with a distribution similar to that in primary islets with the αTC1-cells being peripheral around a core of MIN6 cells. These observations suggest that the cell lines retain an appropriate pattern of expression of cell adhesion molecules and are therefore a suitable model in which to study contact-mediated cell–cell interactions between α- and β-cells.

There is considerable evidence that the presence of α-cells in islets can modify β-cell secretory function, but the nature of the α-cell to β-cell interaction is uncertain. Contact-dependent, cell–cell interactions via cell adhesion molecules and/or gap junctions are able to influence insulin secretory responses [e.g. 1, 3, 19] but it is not clear whether direct cell–cell contact with α-cells modulates insulin secretion from β-cells. Furthermore, a variety of intra-islet autocrine and paracrine regulators of β-cell function have been proposed (see 17) but it is difficult to assess the relative importance of these mechanisms in studies using complex primary islets of Langerhans. Our experimental model of constructing in vitro islet-like structures from islet cell lines has distinct advantages in studies of this type. Thus, generating islet-like structures composed entirely of α- and/or β-cells avoids possible influences of the other endocrine and non-endocrine components of a primary islet [17]. Our previous studies using this experimental model have shown that forming islet-like structures influences both secretory [19–21] and proliferative [24] responses, and that cells within a pseudoislet exhibit synchronous oscillations in intracellular Ca^{2+} consistent with electrical coupling through gap-junctions [36]. Although the low hormone content of transformed islet endocrine cell lines sometimes limits their experimental usefulness [31], this feature of the αTC1 cell-line proved an advantage in the present study. Thus, it is unlikely that the αTC1 cells in MIN6/αTC1 pseudoislets will exert any significant glucagon-dependent paracrine effects since αTC1 cells contain (and secrete) less than 1% of the glucagon of primary α-cells. This allows us to dissociate the cell-contact mediated α-cell to β-cell interactions from local α-cell paracrine effects. Indeed, the results of the current

study strongly suggest that paracrine interactions through stimulatory effects of secreted glucagon on the β-cells are the most important mechanism through which α-cells influence β-cell function in a primary islet. Thus, the direct α-cell to β-cell contact in the heterogenous MIN6/αTC1 pseudoislets was sufficient to enable the formation of anatomically-correct islet-like structures, but had no detectable effect on insulin secretory responses to nutrient (glucose) or non-nutrient (PMA) stimuli. In contrast, exogenous glucagon enhanced insulin secretion, as has been reported previously [12, 22, 23], in accordance with a paracrine role for α-cell-derived glucagon in the regulation of insulin secretion. The route of blood flow through the islet may influence the extent of paracrine interactions between islet cells and there is some debate about whether the direction of islet perfusion favours β-cells influencing α-cells, or vice versa [reviewed in 37]. The issue is further complicated by a recent study demonstrating that human islets lack the segregation between different cell types that is seen in rodent islets and in the heterotypic pseudoislets in the present study, suggesting that intra-islet interactions in human islets are independent of the direction of blood flow [38]. Irrespective of blood flow in vivo, our in vitro studies suggest that the enhanced insulin secretory responses reported in the presence of α-cells [3, 23] are most likely caused by paracrine effects of glucagon rather than direct, contact-mediated effects of α-cells on neighbouring β-cells.

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