Configuration of electrofusion-derived human insulin-secreting cell line as pseudoislets enhances functionality and therapeutic utility

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Abstract

Formation of pseudoislets from rodent cell lines has provided a particularly useful model to study homotypic islet cell interactions and insulin secretion. This study aimed to extend this research to generate and characterize, for the first time, functional human pseudoislets comprising the recently described electrofusion-derived insulin-secreting 1.1B4 human β-cell line. Structural pseudoislets formed readily over 3–7 days in culture using ultra-low-attachment plastic, attaining a static size of 100–200 μm in diameter, corresponding to ~6000 β cells. This was achieved by decreases in cell proliferation and integrity as assessed by BrdU ELISA, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, and lactate dehydrogenase assays. Insulin content was comparable between monolayers and pseudoislets. However, pseudoislet formation enhanced insulin secretion by 1·7- to 12·5-fold in response to acute stimulation with glucose, amino acids, incretin hormones, or drugs compared with equivalent cell monolayers. Western blot and RT-PCR showed expression of key genes involved in cell communication and the stimulus-secretion pathway. Expression of E-Cadherin and connexin 36 and 43 was greatly enhanced in pseudoislets with no appreciable connexin 43 protein expression in monolayers. Comparable levels of insulin, glucokinase, and GLUT1 were found in both cell populations. The improved secretory function of human 1.1B4 cell pseudoislets over monolayers results from improved cellular interactions mediated through gap junction communication. Pseudoislets comprising engineered electrofusion-derived human β cells provide an attractive model for islet research and drug testing as well as offering novel therapeutic application through transplantation.
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Introduction

With current advances in cell engineering, insulin-secreting cell-based therapies for future therapeutic intervention in type 1 diabetes have become more promising (Newgard *et al.* 1997, Efrat 1998, Limbert *et al.* 2007). This strategy offers a potentially unlimited source of readily available functionally competent insulin-producing cells for β cell and transplantation research (Efrat 1998, Limbert *et al.* 2007). To date, this approach has mainly focused on use of rodent insulinoma cell lines (Santere *et al.* 1981, McClenaghan *et al.* 1996, Efrat 1998, Limbert *et al.* 2007) due to limited access to functional human islets or derived β-cell lines (Baroni *et al.* 1999, Narushima *et al.* 2005, Gartner *et al.* 2006, Labriola *et al.* 2009, Ravassard *et al.* 2011). However, in addition to species complications, use of rodent cell lines suffers the disadvantage that cells are routinely grown in culture as monolayers that differ fundamentally from the natural aggregation of β cells as islet micro-organs. Such structural organization has a long evolutionary basis, being observed in ancient hagfish (Emdin & Falkmer 1997), suggesting an important role in maintaining the integrity and physiological function of insulin-secreting cells embedded within the exocrine pancreas.

Consistent with this view, it is well established that the dispersal of isolated rodent islets into single cells results in poor insulin secretory responsiveness compared with intact islets (Lernmark 1974, Halban *et al.* 1987, Carvell *et al.* 2007, Tsang *et al.* 2007). By contrast, dispersed islet cells have also been reaggregated in culture into islet-like structures with restoration of cellular architecture and the original insulin secretory properties of intact islets (Hopcroft *et al.* 1985, Halban *et al.* 1987, Squires *et al.* 2000). Thus, the normal β-cell secretory response in islets depends on intra-islet coordinated signaling cross talk and communication with adjacent cells (Meda *et al.* 1979, Halban *et al.* 1987, Palti *et al.* 1996, Vozzi *et al.* 1995, Squires *et al.* 2000, Serre-Beinier *et al.* 2002). This has prompted much research into the formation

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of functional islet-like structures, termed 'pseudoislets', aimed to better understand islet function and provide artificial islets that will function normally when transplanted to an *in vivo* environment (Kelly *et al.* 2011).

Bioengineered rodent cell lines have been used frequently to generate functional pseudoislets. Most work used pure populations of insulin-secreting (MIN6 and BRIN-BD11) cells (Hamid *et al.* 2001, Luther *et al.* 2006, Kelly *et al.* 2010*a*, Reers *et al.* 2011). However, equivalent studies have been performed more recently with either glucagon (alphaTC)- or somatostatin (TGP52)-secreting cells (Ishihara *et al.* 2003, Brereton *et al.* 2006, 2007, Kelly *et al.* 2010*b*,*c*). The resulting rat or murine pseudoislet structures resembled rodent pancreatic islets in both size and morphology (Hauge-Evans *et al.* 1999, Cavallari *et al.* 2007, Kelly *et al.* 2010*a*) and displayed enhanced secretory properties compared with respective monolayer cells as a result of homotypic cellular interactions (Bosco *et al.* 1989, Hauge-Evans *et al.* 1999, Luther *et al.* 2006, Kitsou-Mylona *et al.* 2008, Kelly *et al.* 2010*a*). Thus, although glucagon and somatostatin are thought to be involved in the regulation of β-cell function in native islets, homologous β-cell interactions appear sufficient for normal insulin secretion within pseudoislets (Bosco *et al.* 1989, Hauge-Evans *et al.* 1999, Brereton *et al.* 2006, 2007, Luther *et al.* 2006). This is supported by recent studies using heterotypic pseudoislets comprising murine MIN6, alphaTC, and TGP52, which showed that the presence of α- and δ-cells in the heterogeneous pseudoislets did not potentiate insulin release in response to established secretagogues compared with pseudoislets comprising MIN6 cells alone (Kelly *et al.* 2010*c*). Although various mechanisms and signaling pathways contribute to cell communication, these studies suggest that E-Cadherin and gap junction proteins connexin 36 and 43 are prominent in maintaining secretory function and islet architecture (Meda *et al.* 1979, Vozzi *et al.* 1995, Squires *et al.* 2000, 2002, Calabrese *et al.* 2004, Luther *et al.* 2005, Brereton *et al.* 2006, Carvell *et al.* 2007, Rogers *et al.* 2007, Kelly *et al.* 2010*b*).

In contrast to rodent islets and derived cell lines, little is known about the formation and functional characteristics of pseudoislets derived from human insulin-secreting cells. We have recently described the generation and characterization of electrofusion-derived human insulin-secreting 1.1B4 cell line that exhibits many of the functional attributes of normal β cells (McCluskey *et al.* 2011). This study aims to generate, for the first time, functional human pseudoislets comprising 1.1B4 human β cells that may more appropriately represent a model for human islets. Pseudoislet characteristics including proliferation, expression of E-Cadherin, and other functional genes; insulin content; and secretory responsiveness were compared with monolayer populations. Promotion of three-dimensional cellular communication significantly enhanced insulin secretory responsiveness indicating the importance and utility of this cellular model for further studies.

Materials and Methods

Cell culture

Human insulin-secreting 1.1B4 cell line was generated by electrofusion of normal human islet cells with immortal human PANC-1 cells as described previously (McCluskey *et al.* 2011). 1.1B4 cells (passages 20–35) were routinely maintained in RPMI-1640 tissue culture medium (37 °C, 5% CO_2) containing 11·1 mmol/l glucose and 0·3 g/l of L-glutamine supplemented; with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 0·1 mg/ml streptomycin; these cells are stable in culture for up to 40 passages (McCluskey *et al.* 2011). Maintenance at 11·1 mmol/l glucose in culture is optimal for function of these cells (unpublished observations). 1.1B4 cells have been species authenticated and deposited at the European Collection Agency of Cell Culture (ECACC; Salisbury, UK) from where they are available for research purposes on request.

Pseudoislet formation and dissociation

Cells were seeded at a density of 5×10^4 cells/well into ultra-low-attachment, six-well, flat-bottomed plates (Corning, Inc., NY, USA). Cells were incubated for 7 days in 5 ml/well tissue culture medium during which time the cells came together to form pseudoislets. To create corresponding 1.1B4 monolayers for functional comparison, pseudoislets were dissociated by centrifuging at 10 000 ***g*** for 5 min to produce a cell pellet. The pellet was then resuspended in enzyme-free Hank's-based cell dissociation buffer (Sigma–Aldrich) for 10 min. Cells were gently agitated until a single cell suspension had been created, and simple morphological examination of 1.1B4 monolayers or pseudoislets was made using an Olympus IX51 inverted microscope. The functional properties of monolayers from dispersed pseudoislets were similar to those of routine monolayers.

Cell proliferation and integrity

Cell proliferation in monolayers and pseudoislets was compared using a commercially available chemiluminescent cell proliferation ELISA kit (Roche Diagnostics Ltd.). This measures BrdU incorporation into newly synthesized DNA. The colorimetric 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay (Mosmann 1983) was used to determine the effects of cell-to-cell contact mitochondrial function. Membrane integrity was evaluated by measurement of lactate dehydrogenase (LDH) release using a commercially available Cytotoxic 96 Non-Radioactive Cytotoxicity Assay kit (Roche Diagnostics Ltd.).

RT-PCR

Total RNA was extracted from 3×10^6 monolayer cells or ~750 pseudoislets using a Qiagen RNeasy Mini Kit

Table 1 List of human specific primers for RT-PCR

Gene	Product size (bp)	Primer sequences	Annealing temperature (°C)	No. of cycles
Insulin	144	F: 5′-GGA ACG AGG CTT CTT CTA CAC-3′ R: 5′-ACA ATG CCA CGC TTC TGC-3′	54	30
Glucokinase	130	F: 5′-TGG ACC AAG GGC TTC AAG GCC-3′ R: 5′-CAT GTA GCA GGC ATT GCA GCC-3′	55	30
Glut1	392	F: 5′-TCC ACG AGC ATC TTC GAGA-3′ R: 5′-ATA CTG GAA GCA CAT GCC C-3′	56	30
Connexin 36	256	F: 5′-GCA GCG AGA ACG GTA CT-3′ R: 5′-CTT GGC CCT TGC TGC TGT GC-3′	62	40
Connexin 43	300	F: 5′-GCT ATT GTG AAT GGG GTG CT-3′ R: 5′-CTG CCA AAA TTG GGA ACT CT-3′	62	34
E-cadherin	535	F: 5′-TGG CTG AAG GTG ACA GAG GC-3′ R: 5′-CGT TAG CCT CGT TTC TCC AGG-3′	57	30
GAPDH	198	F: 5′-AAG GTC AAG GTC GGA GTC AAC G-3′ R: 5′-GTT GTC ATG GAT GAC CTT GGC C-3′	60	30

F, forward; R, reverse.

(Qiagen) according to the manufacturer's protocol. RNA was quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop, Wilmington, DE, USA). Transcriptional gene expression related to β-cell function, gap junction proteins, and E-Cadherin was determined by RT-PCR. The reactions were performed with the one-step RT-PCR kit (Invitrogen) on a Bio-Rad gene cycler using 100 ng total RNA as template. The sequences of the human primers, the sizes of PCR products, cycles, and annealing temperature for each pair are listed in Table 1. PCR products were run on a 2% ethidium bromide agarose gel. Images taken with the Kodak Imagedok program and band density was quantified by a gel imaging densitometer (Bio-Rad Laboratories).

Western blot analysis

1.1B4 cell monolayers and pseudoislets were extracted, and 50 μg protein was loaded and separated on an acrylamide gel by SDS–PAGE and then transferred to nitrocellulose membranes (Invitrogen). Membranes were blocked in 1% skimmed milk and incubated with primary antibodies specific for insulin (mouse monoclonal, AbD Serotec, Oxford, UK), GLUT1 (rabbit polyclonal, Santa Cruz Biotechnology), glucokinase (rabbit polyclonal antibody, S Lenzen, Hanover, Germany) diluted 1:200, gap junction proteins connexin 36 (1:100, rabbit polyclonal, Invitrogen), connexin 43 (1:100, rabbit polyclonal, Sigma–Aldrich), E-Cadherin (1:200 rabbit polyclonal, Transduction Laboratories, Lexington, KY, USA), and GAPDH (1:2000 rabbit polyclonal, Abcam, Cambridge, UK) before detection with HRP-labeled conjugate secondary antibody (ECL-linked anti-rabbit or anti-mouse, Amersham) diluted 1:5000 in PBS–Tween (0·1%). Protein expression was visualized using the Amersham ECL detection system and densitometry of western blot was quantified using ImageJ Gel Analysis Software.

Insulin content and secretion

Cellular insulin content was measured following overnight extraction at 4 °C with acid–ethanol (1·5% (v/v) HCl, 75% ethanol, and 23·5% H_2O). Acute static 20 min incubations were performed to assess insulin secretion using 1.1B4 cell monolayers or pseudoislets as described elsewhere (Kelly *et al*. 2010*a*, McCluskey *et al*. 2011). Low and high glucose concentrations were used to assess the functional responses of these cells. Briefly, monolayers were seeded into 24-well plates (Iwaki Glass, Funabashi, Japan) at a density of 1×10^5 cells/well and incubated overnight to allow cellular attachment. Pseudoislets were picked using a fine glass pipette in the morning of the experiment and placed into Eppendorf tubes at a density of five pseudoislets per tube. For acute test, following incubation at 37 °C in Krebs-Ringer bicarbonate buffer (KRBB) with additions as indicated in the Figures, aliquots were

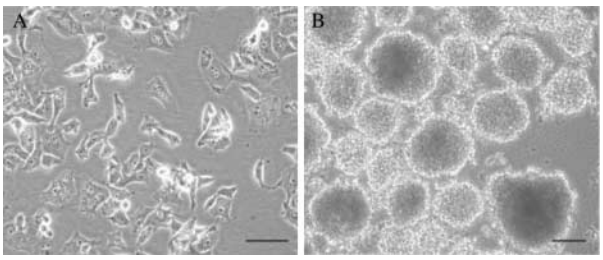

Figure 1 Morphology of 1.1B4 cell monolayers and pseudoislets. Phase-contrast microscopy showing the morphology of 1.1B4 cells as monolayers (A) or pseudoislets (B) after 7 days of culture in suspension in ultra-low-attachment flat-bottomed six-well plates. 1.1B4 cell monolayers were routinely cultured in RPMI-1640 tissue culture medium supplemented with 10% FBS. Images were viewed using Olympus IX51 microscope, ×20 magnification. Scale bar 20 μm for A and 100 μm for B.

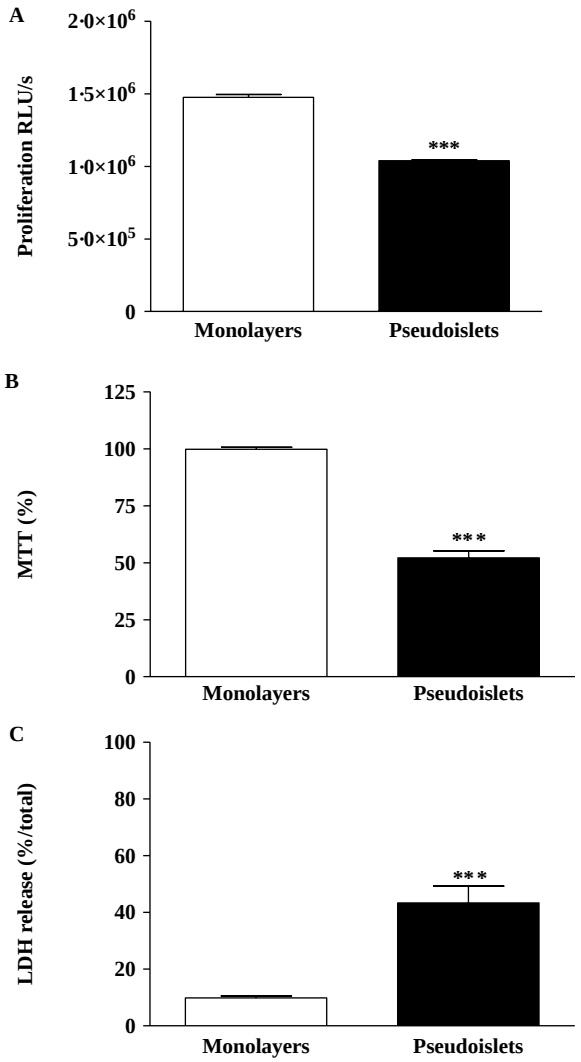

Figure 2 Proliferation and integrity of 1.1B4 cell monolayers and pseudoislets. Cellular proliferation determined by BrdU labeling (A); mitochondrial integrity assessed by MTT assay (B); and membrane integrity evaluated by LDH release (C). These results are presented as percentage of control monolayers (B) and percentage of total releasable LDL (C) respectively. Values are mean±S.E.M. ($n=6$ with each experiment repeated three times independently). ***$P<0·001$ compared with monolayers. RLU, relative light units.

removed and stored at −20 °C until determination of insulin by RIA (Flatt & Bailey 1981), using a human insulin standard.

Statistical analysis

Data are presented as mean±S.E.M. for a given number of observations (n). Differences between two groups of data were assessed using unpaired Student's t-test (GraphPad Prism Software, USA), with statistical significance being considered where $P<0·05$.

Results

Morphology of 1.1B4 cell monolayers and pseudoislets

Figure 1 shows images of 1.1B4 cells in monolayer and pseudoislets formations. The clustering of single cells into pseudoislets occurred as early as day 3. By 7 days, virtually all suspended cells adhered to each other to form pseudoislets (Fig. 1B). These structures were ∼100–200 μm in diameter, with a morphology and size similar to isolated human islets (Lehmann *et al.* 2007). Pseudoislets were dispersed into single-cell suspensions before counting; each pseudoislet comprised 6000±417 cells after 7 days of culture ($n=30$).

Cell proliferation and integrity

Compared with 1.1B4 cell monolayers, pseudoislet formation resulted in a 30% decrease ($P<0·001$) of cell proliferation as assessed by BrdU incorporation (Fig. 2A). MTT assay further indicated that this was accompanied by a 48% reduction ($P<0·001$) in mitochondrial integrity compared with monolayers (Fig. 2B). Similarly, LDH release was increased by 4·4-fold ($P<0·001$) compared with corresponding

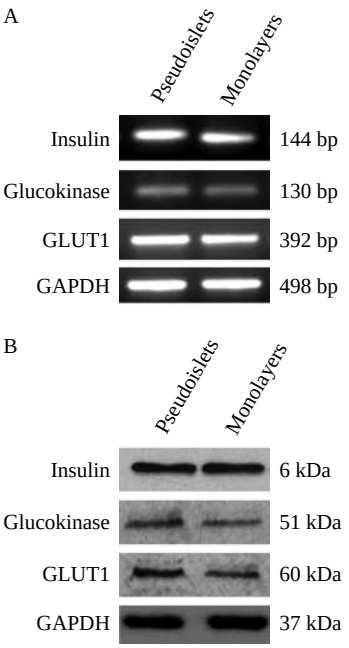

Figure 3 RT-PCR and western blot analysis of insulin, GLUT1, and glucokinase expression. RT-PCR (A) and western blot analysis (B) of insulin, GLUT1, and glucokinase in 1.1B4 cell monolayers and pseudoislets. In (A) *GAPDH* was used as a housekeeping gene, the relative intensity of the each gene product was normalized to GAPDH by densitometry, revealing no significant differences between groups. In (B) GAPDH was used as an internal loading control, and no significant differences of protein signals were detected between groups. Each experiment was repeated three times independently.

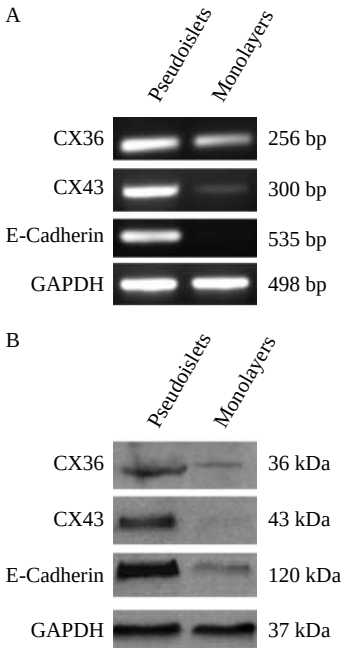

Figure 4 RT-PCR and western blot analysis of Cx36, Cx43, and E-Cadherin (ECAD) expression. RT-PCR (A) and western blot analysis (B) of Cx36, Cx43, and ECAD expression in 1.1B4 cell monolayers and pseudoislets. *GAPDH* was used as a housekeeping gene. In (A) the relative intensity of the each gene product was normalized to GAPDH by densitometry, revealing differences between pseudoislets and monolayers for Cx36, Cx43, and ECAD, $P<0{\cdot}05$–$0{\cdot}001$. In (B) GAPDH was used as an internal loading control, densitometry analysis revealing differences between pseudoislets and monolayers for all three proteins (all $P<0{\cdot}001$). Each experiment was repeated three times independently. Cx36, connexin 36; Cx43, connexin 43; ECAD, E-cadherin.

monolayers (Fig. 2C). Constituent cells appeared healthy with no signs of cellular necrosis even after 7 days of culture.

Functional β-cell gene and protein expression

As shown in Fig. 3A, monolayers and pseudoislets showed comparable levels of mRNA for insulin, glucokinase, and GLUT1 transporter expression. Consistent with this, 1.1B4 cells displayed similar expression of these proteins by western blot analysis, irrespective of structural configuration (Fig. 3B). As illustrated in Fig. 4A, RNA expression of connexin 36, connexin 43, and E-Cadherin appeared higher in pseudoislets than monolayers ($P<0{\cdot}05$–$0{\cdot}001$). Western blot analysis revealed that all three proteins were appreciably more abundant in pseudoislets compared with corresponding monolayers ($P<0{\cdot}001$; Fig. 4B).

Cellular insulin content and glucose-induced insulin release

Insulin content of 1.1B4 cell monolayers and pseudoislets was similar following 7 days in culture (Fig. 5A). The acute effects (20 min) of glucose on insulin secretion are shown in Fig. 5B. Both cell populations displayed stepwise concentration-dependent increases of insulin release ($P<0{\cdot}05$–$0{\cdot}001$), with significantly enhanced responses from pseudoislets (two to five times greater increase than corresponding monolayers; $P<0{\cdot}001$). The insulin output from pseudoislets was consistently greater (1·7 to 2·4-fold; $P<0{\cdot}001$) than equivalent monolayers.

Effects of other nutrients and secretagogues on insulin release

At 16·7 mM glucose, leucine, alanine, arginine, and α-ketoisocaproic acid (KIC) each stimulated insulin release from 1.1B4 monolayers by 1·3-, 2·2-, 1·2-, and 1·3-fold ($P<0{\cdot}01$–$0{\cdot}001$), respectively, compared with glucose alone (Fig. 6A). Cellular arrangement as pseudoislets greatly enhanced these responses ($P<0{\cdot}001$), with insulin output attaining values 5·7- to 12·5-fold greater than monolayers ($P<0{\cdot}001$). Similarly, the gut hormones, gastric inhibitory polypeptide (GIP), glucagon-like peptide 1 (7–36) amide (GLP-1), and cholecystokinin-8 (CCK-8) significantly

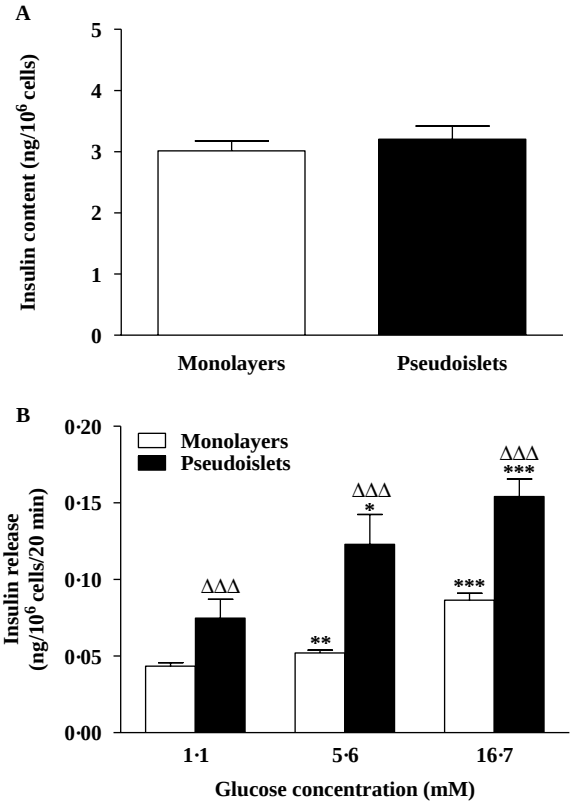

Figure 5 (A) Cellular insulin content and glucose-induced insulin release. Cellular insulin content (A) and glucose-induced insulin release (B) from 1.1B4 cell monolayers and pseudoislets. Values are mean ± S.E.M. ($n=6$ with each experiment repeated three times independently). *$P<0{\cdot}05$, **$P<0{\cdot}01$, and ***$P<0{\cdot}001$ compared with 1·1 mM glucose. $^{\Delta\Delta\Delta}P<0{\cdot}001$ compared with corresponding monolayers.

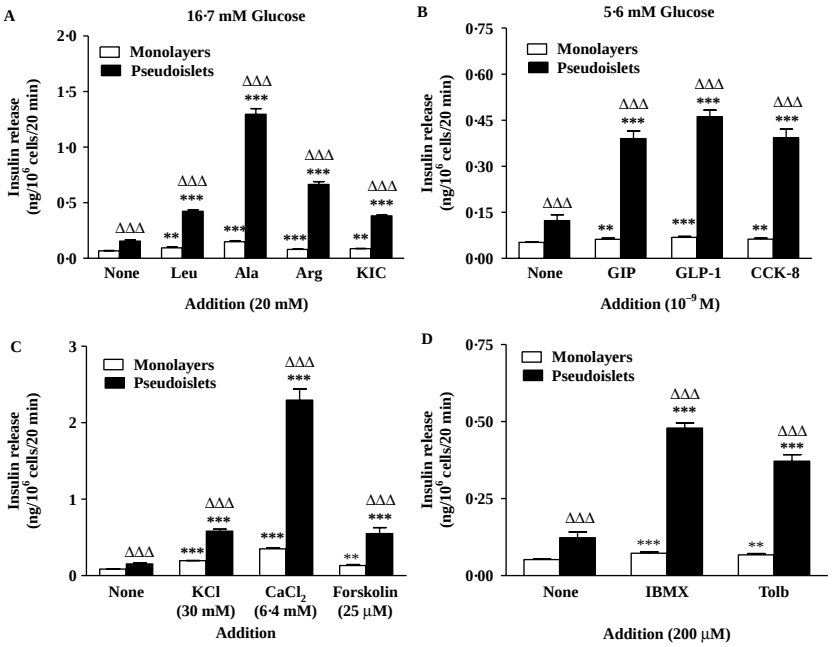

Figure 6 Effects of nutrients, hormones, and drugs on insulin release from 1.1B4 cell monolayers and pseudoislets. Values are mean ± S.E.M. ($n=6$ with each experiment repeated three times independently). $^{**}P<0{\cdot}01$ and $^{***}P<0{\cdot}001$ compared with control (none). $^{\Delta\Delta\Delta}P<0{\cdot}001$ compared with corresponding monolayers. None, 16·7 mmol/l glucose alone (A and C) or 5·6 mmol/l glucose alone (B and D); Leu, leucine; Ala, alanine; Arg, arginine; KIC, 2-ketoisocaproic acid; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1 (7–36) amide; CCK-8, C-cholecystokinin-8; KCl, potassium chloride; $CaCl_2$, calcium chloride; IBMX, 3-isobutyl-1-methylxanthine; Tolb, tolbutamide.

stimulated insulin release ($P<0{\cdot}01$–$0{\cdot}001$) with substantially increased responses from pseudoislets ($P<0{\cdot}001$; Fig. 6B). Extension of these observations to second messenger pathways showed that a depolarizing concentration of KCl, raised extracellular Ca^{2+}, forskolin, 3-isobutyl-1-methylxanthine (IBMX), and the K-ATP channel blocker tolbutamide each stimulated insulin release ($P<0{\cdot}01$–$0{\cdot}001$) from 1.1B4 cells with particularly prominent responses from pseudoislets in comparison with monolayers (Fig. 6C and D).

Discussion

Insulin-releasing 1.1B4 cells represent a new cell line derived from electrofusion of human pancreatic β cells with immortal human epithelial PANC-1 cells (McCluskey *et al.* 2011). This study confirmed features of gene expression and insulin secretion in 1.1B4 cell monolayers reminiscent of normal β cells. These monolayer cells also expressed E-Cadherin and connexins, suggesting an intact innate system for cell-to-cell contacts and communication. Consistent with this view, 1.1B4 cells readily formed pseudoislets during 7 days of suspension culture, apparently mimicking the appearance of human islets, being 100–200 μm in diameter comprising about 6000 individual cells.

Previous studies have shown that formation of rodent pseudoislets was associated with increased E-Cadherin expression, apparently contributing to the attainment of static size in culture (Carvell *et al.* 2007, Hodgkin *et al.* 2007, Kelly *et al.* 2010*a*). E-Cadherin expression, judged by RT-PCR and western blot, was markedly enhanced in 1.1B4 cell pseudoislets compared with monolayers. This was accompanied by a significant reduction in cell proliferation as measured by BrdU incorporation. Interestingly, mitochondrial and membrane integrity assessed by MTT and LDH assays were also decreased, supporting the idea that control of pseudoislet size was partly attributable to increased apoptosis as well as restrained cellular proliferation. However, some degree of hypoxia in the central core of pseudoislets also possibly contributed to these changes in cellular integrity. Nevertheless, the secretory data may be explained by speculating that the majority of cells exhibit enhanced functional activity when configured as pseudoislets rather than as monolayers.

Connexin 36 is believed to play an important role in mediating efficient insulin secretion from pancreatic islets (Hauge-Evans *et al.* 1999, Serre-Beinier *et al.* 2000, Calabrese *et al.* 2004, Bavamian *et al.* 2007, Carvell *et al.* 2007, Rogers *et al.* 2007). In accordance with this, expression of both connexin 36 and connexin 43 proteins was greatly increased

by establishment of three-dimensional structures and individual interactions between pseudoislet cells. Indeed, 1.1B4 cell monolayers exhibited particularly low levels of connexin 43, which may reflect limited cell-to-cell contact, poor gap junction communication, and the relatively weaker glucose responsiveness of monolayers of 1.1B4 cells and rodent β-cell lines compared with native islets (Halban *et al.* 1987, Vozzi *et al.* 1995, Hauge-Evans *et al.* 1999). We cannot rule out that changes in connexin 43 or E-Cadherin also contribute to improved functional activity of pseudoislets, although there is some evidence that connexin 43 may not couple β cells *in vivo* (Bosco *et al.* 2011).

Although formation of 1.1B4 pseudoislets did not affect insulin gene expression or cellular hormone content, basal insulin release and secretory responsiveness to glucose were significantly enhanced compared with simple monolayers. These observations are broadly similar to those reported for pseudoislets derived from rodent cell lines (Luther *et al.* 2006), although glucose sensitivity in this study appears robust. Interestingly, the human GLUT1 islet glucose transporter and glucose-sensor enzyme glucokinase were similarly expressed in 1.1B4 cells irrespective of cellular environment, suggesting the importance of other factors such as improved cell-to-cell communication in the heightened responsiveness of pseudoislets. Early experiments involving electrophysiological measurements or gap junction-mediated diffusion of microinjected dyes in stimulated islets provide ample evidence for transfer of electrogenic signals or important second messengers between islet cells (Meda *et al.* 1986, Howell *et al.* 1994, Palti *et al.* 1996).

Recent studies have shown that 1.1B4 cell monolayers exhibit intact insulin secretory responses to a wide range of nutrients, hormones, drugs, and agents known to trigger specific signaling pathways in pancreatic β cells (McCluskey *et al.* 2011). This study confirms and extends these basic observations by examining the functional impact of configuration of 1.1B4 cells as pseudoislets. Irrespective of the stimulus used, the insulin secretory response of monolayers was greatly amplified by pseudoislet formation, although effects remained inferior to primary human islets and equivalent or less than several rodent cell lines. This involved many established insulin secretagogues selected to activate diverse mechanisms leading into the secretory cascade, which culminates in exocytosis (Ashcroft *et al.* 1994, Aizawa *et al.* 1998). These principally included generation of ATP (leucine/KIC); blockade of K-ATP channels (tolbutamide); direct or Na^+ co-transport-mediated membrane depolarization (arginine/KCl or alanine); direct elevation of intracellular Ca^{2+} (elevated extracellular Ca^{2+}); activation of adenylate cyclase, cyclic AMP, and protein kinase A (GIP/GLP-1/forskolin/IBMX); stimulation of phospholipase C, protein kinase C, and IP_3 (CCK-8); and lastly the triggering of other emerging pathways (GIP/GLP-1/CCK-8).

The global nature of the benefits for insulin secretion of configuration of 1.1B4 cells as pseudoislets points to a fundamental role of three-dimensional cell-to-cell communication in human β-cell function, which is supported by observations with disrupted human islets (Tsang *et al.* 2007). However, these data also suggest that homotypic pseudoislet interactions may be largely sufficient for integrated insulin secretion from human-derived β cells, despite the obvious lack of islet glucagon- or somatostatin-producing cells. Thus, although observations using pseudoislets comprising mouse MIN6, alphaTC1.9, and TGP52 cells support a positive effect of heterotypic cellular interactions, these might be much less significant than originally suspected. Thus, in terms of possible future cell therapy of diabetes, it may be possible to further contemplate transplantation of immunoprotected pseudoislets solely comprising engineered human insulin-secreting cells.

In conclusion, configuration of electrofusion-derived human insulin releasing 1.1B4 cells as pseudoislets greatly enhanced insulin secretory responsiveness and functional gene expression. Such pseudoislets may provide an attractive model for extension of knowledge on the regulatory mechanisms underlying insulin secretion and the survival of native human pancreatic β cells.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

H G-P, J T M, C K, M H H, N H M, and P R F contributed to the conception, design, analysis of data, and intellectual content. H G-P and C K conducted experimental work. H G-P, P R F, J T M, and C K wrote and revised the manuscript. All authors gave final approval.

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