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# Vectorial insulin secretion by pancreatic β-cells

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Morphological studies of pancreatic β-cells have suggested the presence of discrete sensory and secretory domains. In the present study we now provide functional evidence by demonstrating polarity of insulin release by HIT-T15 cells. A significant diffusion barrier across a twin chamber culture system was verified in the presence of confluent HIT-T15 cells. When stimulated with sulphonylurea, ionophore or high potassium, insulin was preferentially released into the lower chamber irrespective of whether secretagogues were added to the upper or lower chambers. Vectorial insulin secretion may be a significant determinant of islet hormone paracrine interactions in the maintenance of glucose homeostasis.

Epithelial polarity; HIT-T15 β-cell/Insulin secretion

## 1. INTRODUCTION

Many integral membrane proteins of epithelial cells, such as receptors or transporters, show an asymmetric distribution on the surface of the cell. In turn, this structural polarity ensures that specific epithelial functions, including transport, absorption and secretion, are vectorial, in being restricted to different surfaces of the cell [1,2]. The establishment and maintenance of epithelial cell polarity is a complex process involving the formation of cell-cell and cell-substratum contacts, and the polarized distribution of membrane and cytoplasmic components. Moreover, it has been suggested that many pathological states may be associated with a breakdown in polarity [3].

Diabetes mellitus is characterized by an inability to maintain glucose homeostasis as a result of partial or absolute insulin deficiency. Insulin is secreted from pancreatic β-cells, which comprise 60–80% of the endocrine cells found in the islet of Langerhans. Islets also contain α- and PP cells, secreting glucagon and pancreatic polypeptide respectively, and δ-cells, which secrete somatostatin. The anatomical relation between the individual cell types and the islet vasculature will clearly be critical in determining the physiological relevance of any islet paracrine effects. At present, although the interaction between the various islet cell types and their specific hormones remains unclear, there is compelling morphological evidence suggesting that insulin-secreting β-cells may be polarized into distinct sensory and secretory domains [4–11]. However, to date, there have been no confirmatory reports documenting the functional polarization of insulin secretion.

The introduction of polycarbonate filter inserts has made it possible to culture epithelial cells in a more physiologically appropriate environment, in which medium has access to both apical and basolateral surfaces of the cell. These twin chamber systems have been widely used in studies of structural and functional specialization to include demonstrations of (i) the polarization of hormone secretion in Sertoli cells [12,13], (ii) receptor-directed polar lymphokine release by helper T cells [14] and (iii) a variety of vectorial functions in the Madin-Darby Canine Kidney (MDCK) cell line, such as transcytosis of growth factors [15], and the sorting of sphingolipids [16] or secretory proteins [17].

In the present study, we have used this culture system to investigate potential bipolar insulin secretion by stimulating clonal HIT-T15 cells [18] with a range of secretagogues. Unlike dispersed adult islet β-cells, HIT-T15 cells can be conveniently grown to a high density in culture and we [19] and others [20,21] have shown that they retain the essential insulin secretory characteristics of isolated islets. Our results demonstrate the vectorial release of insulin by stimulated HIT-T15 cells.

## 2. EXPERIMENTAL

### 2.1 *Materials*

Tissue culture media, sera, antibiotics and plastics were purchased from Gibco Laboratories (New York, NY, USA). [$^{125}$I]Insulin and [$^{3}$H]inulin were from Amersham Corporation (Arlington Hts, IL, USA). Transwell culture inserts were from Costar (Cambridge, MA, USA). Rat insulin standards were from Novo Laboratories (Danbury, CT, USA). All other reagents were purchased from Sigma Chemicals Company (St. Louis, MI, USA).

### 2.2. *HIT-T15 culture*

HIT-T15 cells were generously supplied by Dr A.E. Boyd III

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(Baylor College of Medicine, Houston, TX, USA) and were routinely cultured in RPMI 1640, 10% fetal calf serum (v/v) and antibiotics as we have described [19]. For polarization studies, cells were plated at a density of $4.5 \times 10^6$ into 24.5 mm Transwell inserts containing 1.5 ml of culture medium, and grown until confluent (3–5 days) in 6-well multiwell plates. 2.6 ml of medium was added to the outside of each Transwell in order to achieve equivalent levels of medium between the inside of the insert and the multiwell. Because cell growth was rapid under these conditions, cultures were fed every 12 hours.

2.3. *Monolayer permeability*

The diffusion of [$^{125}$I]insulin and [$^3$H]inulin in the presence or absence of HIT-T15 cells was used to check for the presence of a significant diffusion barrier. Isotopes were added separately to either the upper or lower chamber, and at timed intervals, 200 $\mu$l aliquots were removed from the opposite chamber and counted in the appropriate detector. This procedure was repeated, in triplicate, for each isotope, for each passage of HIT-T15 cells examined.

2.4. *Polarization studies*

On the day of the experiment, Transwell cultures were washed with normal culture medium, transferred to fresh multiwells, and then preincubated for 1 h in glucose-free medium. Cells were washed again and then incubated in culture medium supplemented with glucose (11 mM) and additions as listed in section 3. Secretagogue challenges were added independently to the top or bottom compartment of the culture system. At timed intervals, equivalent aliquots of media were removed from both compartments, centrifuged briefly to pellet any floating cells, and the supernatant stored at −20°C prior to insulin assay. Insulin release was measured by enzyme-linked immunosorbent assay (ELISA) [22].

2.5. *Statistics*

Triplicate Transwell inserts were used for each test condition. All experiments were repeated using different passages of HIT-T15 cells. Data were analysed by analysis of variance (ANOVA) or the *t*-test for paired observations.

## 3. RESULTS

### *Monolayer permeability*

In order to verify that the experimental system was appropriate for defining polarity of $\beta$-cell insulin release, the diffusion of labelled insulin across the Transwell membrane was measured in the presence or absence of confluent HIT-T15 cells. Fig. 1A shows that over a 20 min incubation period, confluent (3–5 days culture) HIT cells presented a significant ($P<0.001$) barrier to the diffusion of [$^{125}$I]insulin across the membrane. In subsequent passages of HIT-T15 cells, a diffusion barrier was consistently observed between 5 and 20 min (not shown). Further, for each passage of cells, similar results were obtained with [$^3$H]inulin (Fig. 1B). In all of the monolayer permeability experiments, similar results were obtained irrespective of whether isotopes were added to the upper or lower chamber.

### *Basal insulin release*

Fig. 2 shows basal HIT-T15 insulin secretion in the Transwell system. Over a 20 min incubation period, insulin was released in a time-dependent manner into both chambers, and there was no significant difference in the levels secreted into each chamber. When the rate of diffusion of labelled insulin (Fig. 1A) is considered relative to the rate of basal insulin secretion (Fig. 2) it is clear that, in the presence of confluent HIT-T15 cells, the rate of diffusion of released insulin is slow compared to the rate at which insulin is secreted into the different compartments. Further, unless the experiments exceeded 20 min or more, mixing of the contents of the upper and lower compartments was negligible.

### *Stimulated insulin release*

Insulin secretion was potentiated by the addition of glibenclamide (20 $\mu$g/ml), A23187 (4 $\mu$M) or high potassium (40 mM). Fig. 3 shows that, in each case, insulin was preferentially released into the lower chamber, irrespective of whether the secretagogue was added to the upper or lower chamber. The ionophore A23187 potentiated insulin secretion by up to 26-fold over basal, insulin levels being between 6–12-fold higher in the lower chamber compared to the upper chamber (Fig. 3A). The sulphonylurea glibenclamide

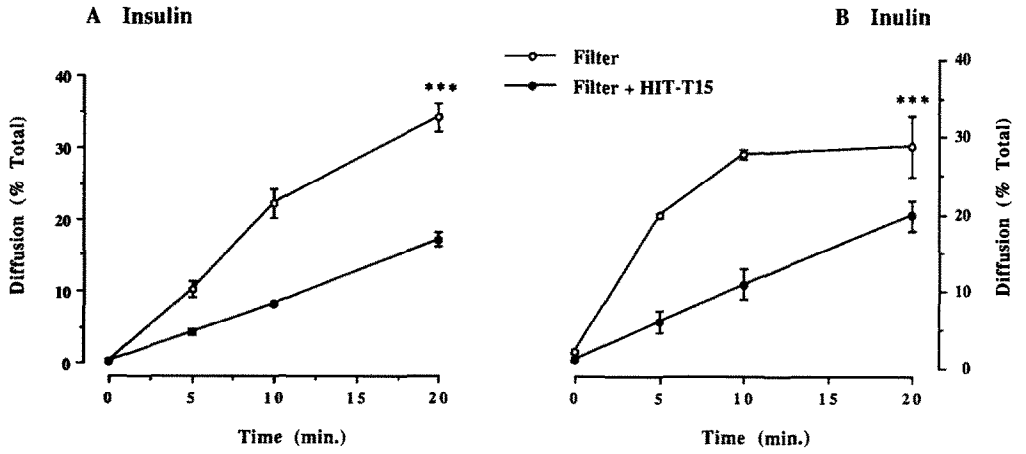


Fig. 1. Confluent HIT-15 cells present a significant (***, $P<0.01$ (ANOVA)) diffusion barrier to [$^{125}$I]insulin (A) or [$^3$H]inulin (B). Label was added to the upper chamber at time '0' and aliquots removed from the lower chamber at timed intervals. Results are expressed as percentage total counts recovered in the lower chamber. Data are given as mean ± SE ($n=3$).

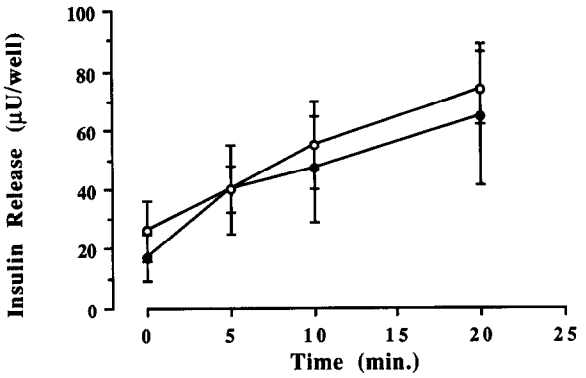


Fig. 2. Basal insulin secretion in HIT-T15 cells is non-polar. Under basal conditions, there was no significant difference in the amounts of insulin secreted into the upper (open circles) or lower (closed circles) chambers. Data are given as mean ± SE ($n=7$).

(Fig. 3B) induced a similar response to A23187: basal insulin release was potentiated by up to 17-fold and insulin levels were between 7–11-fold higher in the lower chamber. Finally, raising the potassium ion concentration to 40 mM (Fig. 3C) evoked an exceptional 70-fold stimulation of insulin release over controls. Insulin

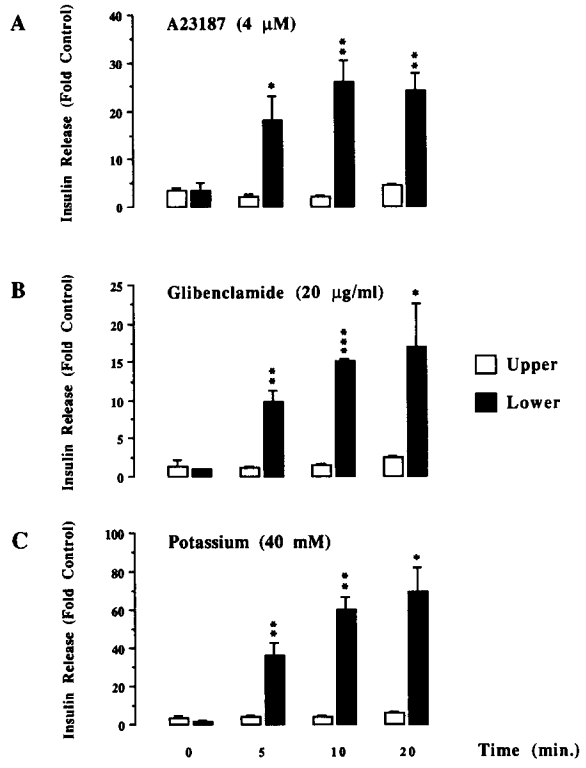


Fig. 3. Polarized secretion of insulin induced by secretagogues. Ionophore (A23187 : A), sulphonylurea (glibenclamide; B) or increased extracellular potassium ion concentrations (C) all significantly potentiated insulin secretion vectorially into the lower (closed circles) chamber, irrespective of whether secretagogue was added to the upper or lower chamber. Data are given as mean ± SE ($n=3$). (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; vs appropriate controls (*t*-test)).

levels were between 10–17-fold-higher in the lower chamber in response to increased potassium.

## 4. DISCUSSION

In this study we have demonstrated vectorial secretion of insulin by clonal HIT-T15 β-cells. This is the first demonstration of functional polarization of the pancreatic β-cell, as predicted by previous ultlrastructural studies.

Early morphological studies suggested functional polarization in that insulin secretory granules appeared to be asymmetrically concentrated towards the 'capillary pole' of the islet [5]. In addition, two studies by Orci and colleagues [9,10] support the hypothesis that the β-cell plasma membrane is polarized into specific domains. First, the asymmetrical emergence of budding RNA viruses was observed from different regions of infected islet monolayer cultures. Polarity of budding is a consequence of the asymmetrical insertion of viral envelope glycoproteins into distinct plasma membrane domains, and it was suggested that pancreatic islet cells might also possess the ability to both sort out and target viral proteins to specific plasma membrane domains [9]. More recently, the liver-type glucose transporter (GLUT2) was shown to be present in the β-cell and localized to specific domains of the plasma membrane, characterized by the presence of microvilli which exclusively project into the intercellular space between adjacent endocrine cells [10]. This implies that glucose uptake will be preferentially restricted to the basolateral regions of the β-cell membrane, rather in those regions closely associated with the capillary lumen.

In situ morphological evidence of structural and functional polarization of β-cells has been provided by the elegant studies of Bonner-Weir who showed that β-cells have two capillary faces [4–6]. Partial degranulation of β-cells by prior in vivo treatment with sulphonylurea, revealed a pronounced polarity of insulin secretory granules towards the pole of the cell facing the central capillary (arbitrarily designated the apical face). This suggests that insulin may also be secreted from specialized plasma membrane domains, the apparent polarity being masked by the intensive granulation present throughout the normal islet [5]. In a further study using freeze fracture replicas, canaliculi were found to extend from one capillary face to the other along the interface of three or more β-cells [6]. Each canaliculus is a channel, closely associated with desmosomes, and across which the microvilli of adjacent cells make contact. The discovery that microvilli are enriched in glucose transporters [10] suggests that the canaliculi may represent sensory domains of the β-cell. However, exocytotic figures were only seen on smooth lateral surfaces and on the pericapillary faces, and may alternatively depict secretory domains [6].

Many epithelial cells form more highly differentiated monolayers when grown on permeable supports in which the basolateral surface is directly accessible to the growth medium, and this is reflected in increased responsiveness to hormones [3]. This was also apparent in the present study with HIT-T15 cells in the Transwell system, since the levels of insulin released on stimulation with secretagogues were both more rapid and larger than previously recorded in standard plastic multiwell dishes. Further, the degree of stimulation of insulin relative to controls was also augmented.

In order to demonstrate polarization, it was important to assess the monolayer permeability in the presence of HIT-T15 cells, and demonstrate a significant diffusion barrier to insulin during the course of the experiment. This was shown using both labelled hormone or inulin. Basal insulin release was similar in both chambers. However, when stimulated with secretagogues insulin secretion was vectorial and was preferentially released into the lower chamber, irrespective of whether the secretagogue was added to the upper or lower chamber. The three secretagogues chosen all stimulate insulin release by causing an increase in intracellular calcium, but achieve this through distinct mechanisms. The ionophore A23187 selectively facilitates calcium entry. The sulphonylurea, glibenclamide, is thought to bind to the ATP-sensitive potassium channel, thereby inducing depolarization of the β-cell membrane and calcium influx [23]. Raising the extracellular potassium concentration directly induces membrane depolarization and calcium influx. High potassium levels evoked the greatest increment of both insulin release and degree of polarization in HIT-T15 cells.

The contribution of polarized β-cell function to the endocrine or paracrine function of the pancreas is at present unknown. It is possible that a failure in the processes involved in establishing β-cell polarity or alternatively, a breakdown in polarity secondary to β-cell stress, may contribute to impaired glucose homeostasis and the development of diabetes. Further studies using this system will help to define the role of establishing and maintaining polarity in normal and pathological β-cell function.

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