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Islet cell interactions with pancreatic B-cells

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Key words. Islets; islet cells; insulin; paracrine effects; junctions.

Introduction

The exquisite glucose-sensitivity of the pancreatic B-cell plays a key role in the hormonal control of glucose homeostasis. The B-cell response is characterized by a rapid discharge of the hormone and by a precise titration of the amount to be released. Its secretory activity is adjusted to other hormonal regulators, so that metabolic demands are quickly met without disturbing other insulin-dependent processes.

A well developed vascular and neural network is thought to rapidly inform the pancreatic B-cells about

the prevailing metabolic conditions. Less clear are the mechanisms involved in the recognition of the various messages, in their integration and their transformation into an insulin secretory response. It is unknown whether these mechanisms are solely located in the pancreatic B-cells or whether they require the participation of other islet cell types. According to a well propagated hypothesis, metabolic and (neuro)hormonal signals are transmitted to the various islet cells which – through intercellular interactions – translate the messages into a finely tuned and coordinated release of pancreatic hormones[87, 90, 100]. The present review is focused upon islet cell interactions with pancreatic B-cells, and pays particular attention to their possible role in generating the essential features of the B-cell response, namely a rapid and metabolically appropriate release of insulin.

A. Indirect interactions

Islet cell products are released in the interstitial space before their discharge into the vascular compartment. Their possible interaction with islet cells can therefore occur in two ways, either with the islet interstitial fluid as sole vector or following their passage in the islet capillary network (fig. 1). Both pathways appear attractive from a physiologic standpoint, in particular in view of the characteristic organization of the various endocrine cell types within the islet of Langerhans[73]. However, their significance might remain hypothetical as long as the islet interstitial space is undefined in terms of composition, flow and compartmentalization. The likelihood of such intra-islet regulation can nevertheless be assessed from a large body of indirect approaches.

1. Local chemical mediators

Most living cells release substances which can act as local chemical mediators on neighboring cells. Several of these agents are rapidly destroyed, cleared or diluted so that their plasma concentration is low and probably physiologically irrelevant. Cell products such as prostaglandins, opioid peptides, biogenic amines and cyclic nucleotides have been detected in the effluent of pancreatic islet cells[19, 22, 46, 84]. Their administration to islets in vitro alters insulin release, which is compatible with a role as local mediators of B-cell function[29, 32, 48, 107]. The main question remains however whether their in vivo concentration in the islet interstitial fluid is sufficiently high to permit effects upon neighboring cells. Correct interpretations also require an identification of their principal source and a description of their acute and chronic effects upon B-cells. Without these clarifications, proposals on local chemical mediators in islets have an all too speculative basis.

2. Pancreatic hormones

2.1 Extracellular concentration in islets

The release of pancreatic hormones in the interstitial fluid creates local concentrations which are far in excess of the levels measured in peripheral plasma or in the vicinity of peripheral receptors. Interstitial hormone

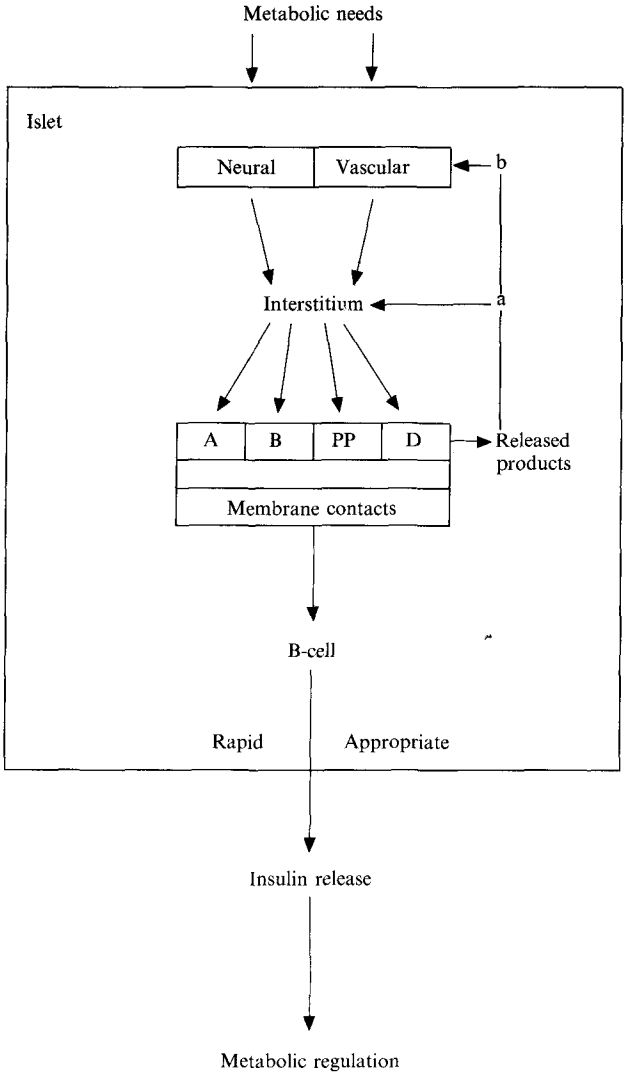

Figure 1. Possible role of islet cell interactions in B-cell function. Islet cells can interact with B-cells either directly via membrane contacts or indirectly via released products. Chemical or hormonal mediators might reach the B-cell immediately after their discharge in the islet interstitium (a) or following passage in the vascular compartment (b). Both types of islet cell interaction could aid the pancreatic B-cell in responding rapidly and appropriately to the metabolic needs.

concentrations are almost impossible to estimate as no information exists on pancreatic hormone distribution in the islet intercellular space. A heterogenous distribution can be predicted from the clustered localization of the B and non-B cell populations[25, 73]; in rat islets for example, a central mass of insulin-containing B-cells is surrounded by a peripheral layer of non-B-cells[25, 73]. Differences in local hormone concentration are also expected when homologous cells vary in secretory activity or when a compartmentalization exists within the intercellular space. Other variables are the distance between the site of hormone release and that of their discharge in the islet capillaries, the flow characteristics of interstitial and vascular fluid, and the properties of endothelial cells and basement membrane. It is evident from these reflections how difficult it will be to gain complete insights in the islet interstitium in vivo. What can be

concluded at the present time, is that marked differences will certainly occur between the intercellular hormone concentrations in the intact pancreas and those present in isolated islets. The role of locally released hormones might thus differ with the type of islet preparation.

Pancreatic hormone concentrations in the islet vascular compartment are somewhat easier to estimate. In the afferent arterioles, the in vivo levels should be similar to those measured in peripheral blood. The levels are expected to increase in the islet capillary network and should be maximal in the collecting venules which leave the islets. These maximal concentrations can be derived from the hormone levels in the pancreatic effluent[8, 16, 33] and the blood flow distribution over endocrine and exocrine pancreas[31, 61]. The values thus obtained (table, see also Weir et al.[106]) lack sufficient experimental support and should therefore be solely considered as an attempt to quantify a frequently cited notion. Extrapolation of these data to the in vivo situation would indicate that rat islet cells are exposed to plasma pancreatic hormone levels varying between 10^{-10} and 10^{-7} M for insulin, between 10^{-10} and 10^{-8} M for glucagon and between 10^{-11} and 10^{-9} M for somatostatin (table). It is obvious that these figures will vary with the size and cellular composition of the islets, with the secretory activity and localization of the various cell types, and with the rate of islet blood flow.

2.2 *Access to target cells*

2.2.1 *Compartmentalization*

Whether paracrine or endocrine, the effects of pancreatic hormones upon B-cells require their access to the target cells. The properties of the islet interstitium are therefore important determinants in the possible intra-islet regulation via release products. According to Kawai et al., the intercellular space in islets is compartmentalized in hormone-rich regions which transfer the released hormone to the venous capillaries, and in hormone-poor regions where circulating hormones can interact with specific receptors[35]; according to the same authors, these compartments are sealed by tight junctions which prevent diffusion of locally released hormones to the receptor-pole of the cells, and which thus maintain the islet cell sensitivity to the low plasma hormone levels[35, 99]. In rat and human pancreas however, in 't Veld et al. were unable to detect tight junctions in islets in situ[28]. In fact, tight junctions were so far only described after mechanical or proteolytic treatment of the pancreatic gland[28, 65, 66], as if they assume their sealing function in conditions of chemical aggression[28]. These observations rather suggest that, if clearly separated interstitial compartments do exist in the normal endocrine pancreas, they should be generated by other structures than tight junctions.

2.2.2 *Blood flow*

Since B- and non-B cells are not randomly distributed but clustered in particular microdomains[25, 73], it is important to know which population is first irrigated before making predictions about possible endocrine routes of intra-islet regulation.

According to Fujita et al., the blood of the islet vas afferens first streams through the non-B cell region and then reaches the B-cell region[13]. A similar pattern was described in adult human islets, where A- and D-cells border the penetrating vessels and surround the more centrally located B-cells[72]. Such irrigation pattern creates an anatomical basis for glucagon and somatostatin to exert immediate endocrine effects upon the pancreatic B-cells[13].

More recently, Bonner-Weir and Orci have re-examined the three-dimensional organisation of the microvasculature in rat islets[6]. In contrast to the earlier observations, the afferent vessels were found to enter the islets in areas devoid of non-B cells and to send their capillaries through the B-cell core before passing through the non-B cell mantle[6]. These results rather indicate that in terms of endocrine regulation, rat pancreatic B-cells are only accessible to pancreatic hormones which have circulated through the organism, but not to those which were just discharged into the blood stream.

2.2.3 *Cellular topography*

The access of pancreatic hormones to islet cells might be considerably facilitated if they are released in the vicinity of their target cells. A morphological relationship between two islet cell types might therefore be physiologically relevant.

A close topographical association has been noted between somatostatin-containing D-cells on one hand and insulin-containing B-cells or glucagon-containing A-cells on the other hand. Examples of this relationship were found throughout the ontogeny and phylogeny of the endocrine pancreas, and were particularly well illustrated in reports on the islet cell distribution in vertebrate species[9, 10, 25, 68, 71, 73].

The preferential association of D-cells with pancreatic B-cells was already recognized more than a decade ago, when it led to the first speculations about insulin release being regulated by a local inhibitory factor, later identified as somatostatin[25, 26]. Even after islet dissociation, many somatostatin-containing D-cells remain attached to B-cells, while almost all glucagon-containing A-cells

Pancreatic hormone levels in the rat

	In vitro perfused pancreas Organ effluent	Islet venous capillary	Basal in vivo Peripheral plasma
Insulin	10^{-10} to 10^{-8}	10^{-9} to 10^{-7}	10^{-10}
Glucagon	0.5×10^{-10} to 1.5×10^{-9}	0.5×10^{-9} to 1.5×10^{-8}	10^{-10}
Somatostatin	10^{-11} to 0.5×10^{-10}	10^{-10} to 0.5×10^{-9}	10^{-11}

The range of hormone concentrations (mol/l) is taken from studies where the three hormones were measured in rat pancreatic effluent[14-16] or in rat peripheral plasma[15]. The levels in islet venous capillaries were calculated from those in the organ effluent, assuming that the islets account for approximately 10% of the total pancreatic blood flow[18].

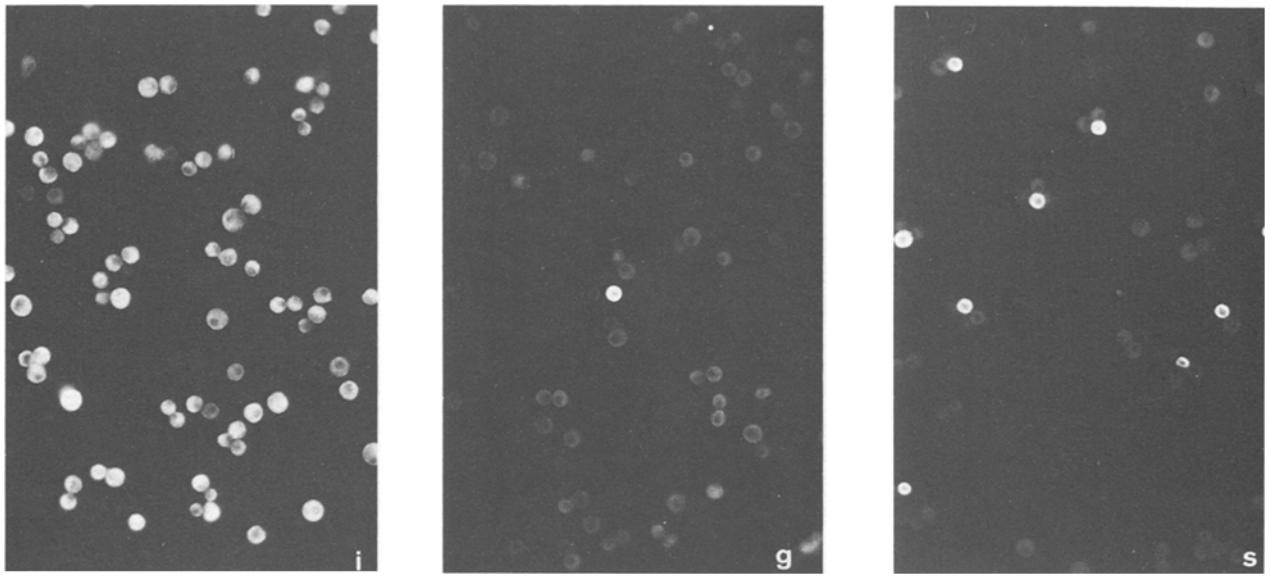

Figure 2. Preferential association of D-cells with pancreatic B-cells after islet dissociation. When single B- and non-B-cells are prepared from islet cell suspensions[77, 101], a cell fraction is left which contains structurally coupled islet cells and which is composed of 90–95% B-cells, less than 2% A-cells and 5–10% D-cells. After fluorescent identification of the pancreatic hormones in this fraction, the rare glucagon-containing A-cells (g) were detected as single units, whereas the more abundant somatostatin-containing D-cells (s) were all structurally coupled to insulin-containing B-cells (i). In unpurified islet cell suspensions, A-cells are 4-fold more abundant than D-cells (not shown).

occur as single cells[76] (fig. 2). This higher coupling tendency of the D-cells has been attributed to their multiple cytoplasmic processes[76], which could – just as in the antral gland[43] – provide an anatomical basis for close and strong contacts with putative effector cells.

The characteristic topography of the pancreatic D-cells might thus facilitate access of somatostatin to a small proportion of B-cells, and can therefore be considered as indirect evidence for paracrine effects in pancreatic islets[68].

2.3 Receptors upon pancreatic B-cells

If pancreatic hormones interact with islet cells, they are expected to do so via membrane receptors. The recognition and characterization of specific binding sites for pancreatic hormones can thus be indicative for the existence of intra-islet regulatory mechanisms. Such investigation faces two technical problems, firstly the need to prepare large cell numbers and, secondly, the risk that endogenously released hormone can markedly reduce the specific activity of the tracer. Despite these obstacles, evidence has been reported in favor of glucagon, insulin and somatostatin receptors in islet cells.

The presence of insulin receptors has been examined in isolated islets[103]. Specific binding of I^{125}-insulin was detected during 20-min incubations of 100-µl samples containing 100 islets. Scatchard analysis was compatible with the existence of high affinity receptors with a dissociation constant of 0.46 nM. Crucial in this study is the assumption that the endogenous insulin levels remained under 50 pM and thus exerted no interference with tracer binding. In support for a biological significance of insulin binding to islet B-cells was the finding that the K_D of insulin binding corresponded to the insulin concentrations which inhibited glucose-induced insulin release from isolated islets[103]. However, it is unclear from the presented binding experiments on which cell type the high affinity receptors for insulin are located. This identification seems important as several investigators have been unable to demonstrate an effect of insulin upon its own release in vitro[21, 49]. To answer this question, autoradiographs were performed on monolayer islet cell cultures which had been exposed to I^{125}-insulin for 60 min at 37 °C[74]. Specific binding of insulin was detected in approximately 50% B-cells, 30% A-cells and 15% D-cells[74]. Unfortunately, no distinction could be made between receptor binding and intracellular incorporation, nor between low and high affinity sites. A better characterization of insulin interactions with islet cells can be expected if binding studies are carried out on purified islet cell preparations. The recent development of islet cell purification techniques[76, 101] has created the possibility to undertake such experiments. Employing this novel approach, Van Schravendijk et al. were unable to detect high affinity insulin receptors upon cultured pancreatic A-cells[102] nor B-cells (unpublished observations).

In the same purified preparations, glucagon receptors were identified upon insulin-containing B-cells (Van Schravendijk et al., unpublished observations). Earlier studies had already demonstrated specific binding of I^{125} glucagon to insulin-secreting tumoral cells[4, 17]. In autoradiographs of monolayer cultures, I^{125} glucagon was found on the surface of and inside A-, B- and D-cells but only 15–37% of the cells interacted specifically with the labeled hormone[74].

Somatostatin receptors have been described in isolated islets and in insulinoma cells[59, 83]. Interestingly, conditions which stimulate insulin release, increased the number of somatostatin receptors[59, 93]. This phenomenon was attributed to receptor recruitment from secretory

vesicles, and led to the hypothesis that hormone release can be modulated by receptor interactions at the junction of secretory vesicles and the plasma membrane[59, 93]. If such mechanism really exists, it might explain the heterogenous labeling of islet monolayers exposed to I^{125} somatostatin[74]. Again, studies on purified islet cells are necessary to confirm the existence of somatostatin receptors on B-cells and to evaluate their role in the regulation of insulin release.

2.4 Effects upon insulin release

2.4.1 Exogenous hormones

Exogenous glucagon stimulates insulin release in vivo and in vitro[20, 48, 85]. The hormone is expected to bind to surface receptors on pancreatic B-cells, thereby stimulating adenylcyclase activity and cyclic AMP production[41, 91, 98].

The administration of somatostatin blocks insulin release under various in vivo and in vitro conditions[1, 37]. The peptide is thought to interact with B-cells, but the mechanism leading to a reduction in hormone release is not yet identified. Both pancreatic polypeptide and insulin have been reported to inhibit insulin release in vivo[44, 64, 104]; the PP-effect has not yet been reproduced in vitro, while in vitro testing of the insulin effect has remained controversial[21, 49, 92, 103].

2.4.2 Endogenous hormones

The marked effects of exogenous glucagon and somatostatin upon insulin release have raised the hypothesis that endogenously released pancreatic hormones modulate the secretory activity of B-cells[68, 86, 89]. Several experimental models have been used to test this possibility and to examine whether such actions are transmitted via paracrine or endocrine routes. It seems essential to discuss these studies according to the experimental model used, since the way islet cells interact will vary with the type of islet cell preparation. Paracrine or endocrine pathways within the endocrine pancreas are indeed likely to undergo profound changes as soon as the normal architecture of the pancreatic organ is disturbed.

In vivo models

Although it is most relevant to know whether insulin release in vivo is regulated by endogenous pancreatic hormones, this condition has been the least well examined. One approach consists in monitoring insulin release while the secretory function of pancreatic A-, D- or PP-cells is selectively suppressed or stimulated. Unfortunately, selective modulators of glucagon, somatostatin or PP-release have not yet been described. It has been argued that – at certain concentrations – the somatostatin analog ALA^5-TRP^8-SS, interacts solely with the pancreatic D-cells thus diminishing somatostatin release without affecting directly the other islet cell types[94]. In this case, the parallel increase in glucagon and insulin levels following the in vivo administration of the analog, might well express an in vivo intra-islet effect of pancreatic somatostatin[94].

Other suggestive evidence for a participation of islet non B-cells in the secretory function of B-cells can be sought in the insulin release characteristics of organisms with an abnormal proportion of islet non-B-cells relative to islet B-cells. Among the animal models of disease, the obese BL/6J mouse represents a suggestive example in this context, as its hyperinsulinemic diabetic state was associated with a reduced number of pancreatic D-cells[2]. This anomaly in the endocrine pancreas could result in a diminished local somatostatin release which would permit an increased secretory activity of A- and B-cells and lead to the disturbed glucose homeostasis which is characteristically observed in this animal strain. The search for similar examples in human diabetes is considerably hindered by the heterogeneity of the disease and by the technical difficulties in analyzing the endocrine pancreas in a sufficient number of patients and age-matched controls. It is thus not surprising that divergent results have been reported on the volume density of the various islet cell types in type 2 diabetics[15, 36, 81]. However, the data do not exclude the possibility that certain forms of non-insulin dependent diabetes are associated with an altered islet composition and thus with a disturbed intra-islet regulation of insulin release.

More experimental support has been collected for a causal relationship between an abnormal cellular composition of the endocrine pancreas and conditions of persistent neonatal hypoglycemia. The hyperinsulinemic state of these infants has been attributed to a reduction in the number of somatostatin-containing cells[5, 11, 24] and could thus result from an unrestrained secretory activity of B-cells. In a detailed analysis of 15 cases, Rahier et al. correlated the hypoglycemic syndrome to the presence of B-cells with larger nuclei; they also reported an increase in the number of PP-cells in addition to the reduced number of D-cells[82]. The hypoglycemic condition might thus be attributed to a hyperactive B-cell population, but it is unknown whether this increased activity is causally related to an altered number of D- or PP-cells.

That endogenously released pancreatic hormones can regulate insulin release in man, is best illustrated in patients with a pancreatic glucagonoma or somatostatinoma. In both conditions, alterations in insulin release have been described and were attributed to an increased and/or anarchic release of tumoral glucagon – or somatostatin-like material[40, 50, 79].

Perfused pancreas

The possible role of endogenous hormones in the regulation of insulin release has been more extensively investigated in perfused pancreas preparations. Most experiments consisted in measuring insulin release under conditions which alter the secretory function of A- or D-cells. In several reports, the observed variations in effluent insulin were explained on the basis of regulatory effects of endogenously released glucagon or somatostatin[8, 87, 89, 90]. Other studies underlined the dissociation between glucagon and insulin release and questioned the existence of a local regulation[51]. The complexity of the endocrine pancreas and insufficient

knowledge about cell specific interactions make it presently extremely difficult to draw firm conclusions from these data.

In terms of insulin release, more suggestive evidence has been collected in support of a regulatory role of endogenous somatostatin than of endogenous glucagon. This notion has been illustrated both for locally released and for circulating somatostatin[33,35]. In the first report, glucagon-induced insulin release became progressively less marked when increasing glucagon concentrations were infused in an isolated rat pancreas[33]. As the reduced secretory activity of the B-cells coincided with a progressive rise in somatostatin release, it was concluded that locally released somatostatin regulates insulin release from the perfused pancreas. In the second study, circulating somatostatin levels – which are far lower than those generated in the endocrine pancreas – were found to inhibit arginine – induced insulin release from the perfused dog pancreas[35]. This observation strongly suggests the existence of a somatostatin-poor islet compartment where low circulating somatostatin levels regulate B-cell function. According to Kawai et al., the infused somatostatin acts directly upon the pancreatic B-cells[35]; however, it was not excluded that the observed reduction in insulin release resulted from a somatostatin-induced inhibition of glucagon release.

Reports suggesting a regulation of insulin release via locally released somatostatin or via plasma somatostatin are not necessarily conflicting. Both routes might be acting in vivo, even without a tight junctional compartmentalization. It is for instance conceivable that one population of B-cells is exposed to the released peptides of neighboring D-cells, whereas another population undergoes control by circulating somatostatin – either directly or via the glucagon-releasing A-cells. Caution is nevertheless required when extrapolating these results to the in vivo situation, as the isolation of the pancreatic organ, its denervation and its in vitro perfusion conditions can disturb paracrine as well as endocrine routes of B-cell regulation.

Isolated islets

For obvious reasons, isolated islets represent an inappropriate model for assessing intra-islet regulatory pathways in vivo. The isolation procedure not only destroys the vascular and neural components, but also profoundly alters flow characteristics and compartmentalization of the islet interstitial space. While the disruption of the in situ architecture is expected to increase paracrine effects, the development of tight junctions during the isolation procedure[28] might trap released products within confined areas, and thus expose islet cell membranes to abnormally high hormone concentrations[66]. Isolated islets have been – and still are – extremely useful in gaining knowledge about B-cell function. It seems therefore interesting to know to which extent insulin release from isolated islets is regulated by paracrine phenomena.

One method consists in measuring insulin release under conditions where endogenously discharged glucagon or somatostatin is neutralized by an excess of antibodies. The validity of this experimental design depends on the antibodies' capability to bind all free hormone before it exerts any biological effect upon neighboring cells. It is not clear whether this criterion is met in all reports; neither has it been excluded that the added glucagon or somatostatin antibodies interact directly with islet cells, as has been observed for insulin antibodies[34] and suggested for glucagon antibodies[95].

Somatostatin antibodies increased glucose-induced insulin release from adult rat islets[30,88,95,96], and from monolayer cultures of neonatal islet cells[14]. These results support the view that insulin release from isolated islets is regulated by locally released somatostatin. Experiments with glucagon antibodies have been less numerous and more controversial as an inhibition of insulin release was observed in one study[88] and a stimulation in two others[14,95]. This discrepancy can result from differences in islet cell composition or in antibody properties, and might be related to the dual effect that glucagon might exert upon insulin release, namely a stimulating one via direct interaction with the B-cell and an inhibitory one via an increased somatostatin release[75,85].

The possibility that glucagon exerts paracrine effects in isolated islets has been better documented by Trimble and Renold, who compared glucose-induced insulin release from glucagon-poor and glucagon-rich islets isolated from the same rat pancreas[97]. This experimental design bears upon the knowledge that islets in the ventral primordium-derived pancreatic tissue contain many more pancreatic polypeptide cells and proportionally less glucagon cells than islets in the dorsal part[3]. The observation that glucose released up to 50% more insulin from dorsal islets than from ventral islets[97] strongly suggests that endogenous glucagon amplifies glucose-induced insulin release from isolated islets as has been proposed 15 years ago[86].

Purified B-cells

The possible regulatory role of locally released glucagon and somatostatin can also be assessed by comparing glucose-induced insulin release from isolated islets with that from purified B-cells[78]. That pancreatic B-cells require intra-islet factors to respond appropriately to glucose is illustrated by the fact that single purified B-cells release 30-fold less insulin than B-cells which are lodged in intact isolated islets[78] (fig. 3). This secretory deficiency of single B-cells was not associated with a reduced rate of glucose transport or oxidation[18], but was related – at least in part – to a deficiency in cellular cyclic AMP levels[77,91]. When glucagon was added to single B-cells, a marked increase was measured in both cellular cyclic AMP and glucose-induced insulin release[77,91]. Interestingly, the stimulatory effect of glucagon upon single B-cells was detected at lower concentrations than when the hormone was added to intact islets (fig. 4). The higher glucagon-sensitivity of single B-cells weakens the argument that cell damage is responsible for their poor secretory response to glucose; it rather suggests that isolated islets are already exposed to endogenously released glucagon which keeps the B-cell cyclic AMP levels higher than in single B-cells without glucagon added. Paracrine effects of glucagon are therefore thought to participate in glucose-induced insulin release from iso-

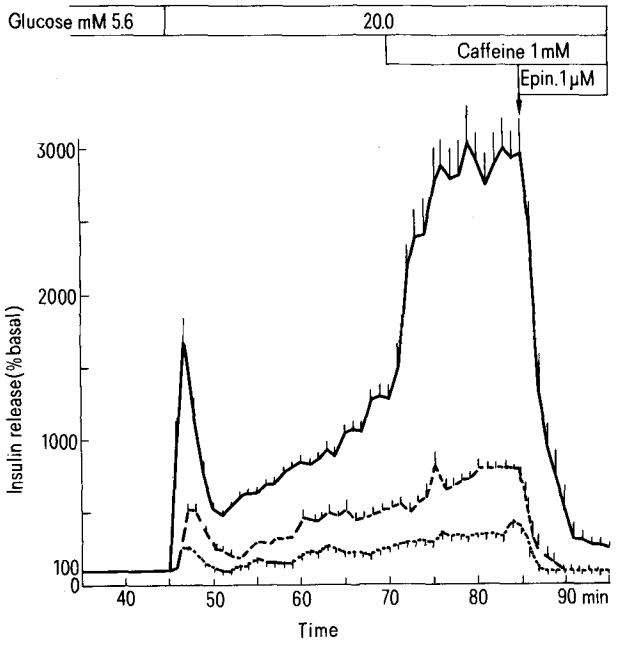

Figure 3. Insulin release from intact islets (solid line, n = 10), dissociated islet cells (broken line, n = 8) and single purified B-cells (dotted line, n = 9). The three fractions were cultured for 16 h prior to perifusion[78]. Results are expressed as percentage of the hormone release at 5.6 mM glucose (basal) and plotted as mean ± SEM. A statistically significant difference was measured between islets and islet cells ($p < 0.001$), and between unpurified and purified islet cell preparations ($p < 0.01$). (Reprinted with permission of Proc. Natl Acad. Sci.; Pipeleers et al.[78]).

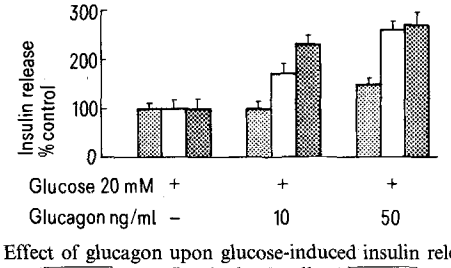

Figure 4. Effect of glucagon upon glucose-induced insulin release from intact islets (▒▒▒, n = 6), single B-cells (□, n = 6) and structurally coupled B-cells (■■■, n = 5). The three fractions were cultured for 16 h prior to the 120-min static incubation. Results are expressed as percentage (mean ± SEM) of the hormone release measured at 20 mM glucose without glucagon added. At 10 ng/ml, glucagon significantly increased ($p < 0.005$) glucose-induced insulin release from purified B-cells but not from intact islets; at 50 ng/ml, glucagon was three-fold more potent in stimulating hormone release from purified B-cells than from intact islets.

lated islets. This notion is further supported by the better secretory activity of B-cells that were mixed with glucagon – containing A-cells[78]. As somatostatin was shown to counteract glucagon-induced cyclic AMP production in single B-cells[91], it is likely that endogenous somatostatin also regulates insulin release from isolated islets.

B. Direct interactions

Neighboring cells can interact via their adjacent membranes. Intercellular contacts are established through adhesion of cell coat molecules or through membrane specializations such as desmosomes, gap and tight junctions. Each of these forms of cell contact have been identified between islet cells[42,65,70] but their functional significance is far from understood. In analogy to their role in other tissues, membrane interactions in islets are thought to coordinate the secretory function of the various islet cells[65,69]. We will first examine whether insulin release varies with the number of islet cell contacts, then discuss how cell contacts could influence insulin release, and finally illustrate that membrane interactions between islet cells can represent another regulatory site via which insulin release can be adapted to physiologic needs.

1. Role in insulin release

While glucose increases insulin release from intact islets 10-fold, its stimulating effect upon dispersed islet cells is much less marked (fig. 3). The decreased secretory activity of dissociated B-cells can be attributed to cellular damage inflicted during their preparation, to diminished paracrine effects due to the immediate dilution of the released hormones or to disruption of cell contacts. The dissociated and purified islet cell suspensions have been extensively tested using various viability criteria[80]. No signs of cell damage were detected[80], which is also reflected in the more than 90% cell survival over a 4-day culture period. The reduced responsiveness of dissociated islet cells seems therefore not caused by cell lesions. It has been partly attributed to a decrease in paracrine effects of glucagon, as outlined in the previous section. Whether the disruption of cell contacts also contributes to the decreased secretory activity appears from insulin release studies on B-cell preparations with a different degree of cell aggregation.

When single purified B-cells are compared to structurally coupled purified B-cells, a markedly higher secretory activity is measured in the aggregated cells[47,77,78]. Both first and second phase insulin release were fourfold higher in the small groups of aggregated B-cells that were isolated as such from the rat pancreas (fig. 5)[78]. A similar difference was noted between hormone release from single B-cells and that from B-cell clumps that were formed by reaggregation of single cells[47,77]. Both in perifusion and in static incubations, basal insulin release from single B-cells was comparable to that from structurally coupled B-cells[47,77,78], indicating that their poor secretory response to glucose originates from a defect in stimulus-secretion coupling rather than from an uncontrolled hormone discharge. As single and structurally coupled B-cells did not differ in glucose handling[18] nor in cyclic AMP levels[91], their different secretory responsiveness might well reside in a different organization of their secretory apparatus.

Studies on unpurified islet cell preparations have also demonstrated a loss in glucose-induced insulin release upon disruption of the pancreatic islet[7,23]. According to Halban et al., this deficient response is related to the lack of single B-cells to restrain their hormone release at low glucose concentrations[23]. In their experiments, islet cells that were reaggregated immediately after dissociation did indeed release less hormone at low glucose levels and markedly more at high glucose. In recent work by Chertow et al., a better secretory activity was also

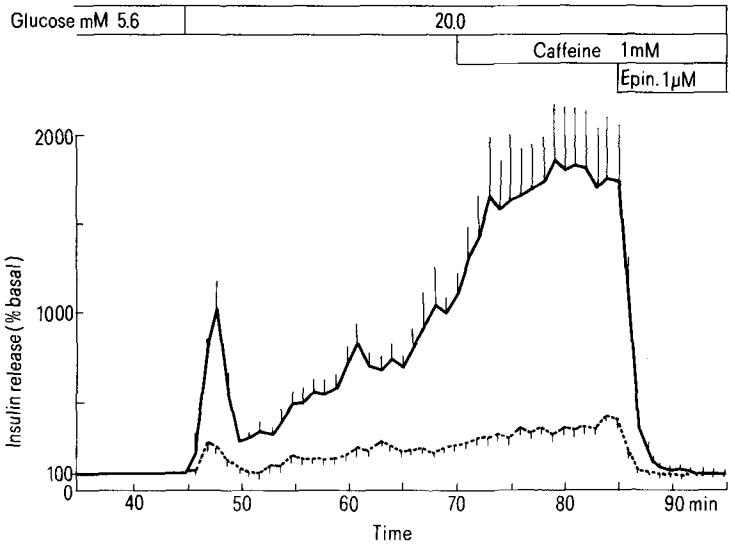

Figure 5. Insulin release from structurally coupled B-cells (solid line, n = 8) and from single B-cells (dotted line, n = 9), Results are expressed as in figure 3. The significance level of the difference with the single B-cell response is $p < 0.001$ for first phase insulin release and $p < 0.02$ for the second phase. (Reprinted with permission of Proc. Natl Acad. Sci., Pipeleers et al.[78]).

observed when the islet cells were reaggregated, but this improvement was not associated with a reduction in basal hormone release[7].

2. *Action mechanism*

Several hypotheses can be developed to explain how intercellular contacts could regulate the insulin release process.

Since the development of contact sites requires an interaction between specific membrane components, it is conceivable that the formation of intercellular contacts is followed by a further organization of adjacent parts of the cells and their membranes. Desmosomes are for example characterized by a symmetrical array of extracellular, membranous as well as intracellular components; junctional complexes develop in specific domains which are associated with microfilaments[63, 105]. One can therefore speculate on the role of intercellular contacts in maintaining a similar organization in adjacent cells and in creating a cell polarity. Whether this occurs in islet cells is unclear. In a study on living mouse pancreas in situ, McCuskey and Chapman found no consistent polarity in the endocrine cells[52].

Membrane contacts can also regulate the function of neighboring cells through direct exchange of ions and small molecules. This form of intercellular communication is thought to proceed via the membrane channels of gap junctions. Gap junctions have been identified in isolated islets, where they were found to connect B-cells to other B-cells as well as to islet non-B-cells[65, 67, 69]. Electrotonic and metabolic coupling between islet cells has also been demonstrated in vitro[38, 39, 56, 57, 58, 60, 62], but the functional significance of this phenomenon is not yet defined. The idea that gap junctions coordinate and synchronize pancreatic hormone release is thus still hypothetical, but attractive.

3. *Regulatory site*

As in other tissues, membrane interactions between islet cells represent dynamic events rather than fixed structures. The formation, magnitude or activity of islet cell contacts can be modulated by agents which are also known to alter insulin release[27, 53, 54, 55, 57, 58, 60]. Since cell contacts have been implicated in the regulation of insulin release, one could wonder whether they can function as regulatory sites in the acute or chronic adaptation of B-cell function.

3.1 *Adhesion*

Glucose as well as dibutyryl cyclic AMP stimulate the reaggregation of single B-cells during short incubations[47] (fig. 6). This effect is not the result of an elevated secretory activity, nor of an increased rate in glucose metabolism, but was highly dependent on extracellular calcium[47]. The newly formed aggregates were easily dissociated by calcium removal, indicating their dependency on adhesive forces. The better secretory activity of the reaggregated B-cells[47] further suggests that an increased intercellular adhesion can participate in the insulin release response to glucose or cyclic AMP. Retinoids were also shown to increase adhesion between islet cells, and were likewise found to amplify the secretory response of B-cells[7].

3.2 *Tight junctions*

Although tight junctions have not been detected in normal islets in situ[28], the islet cell membranes are capable to rapidly develop tight junctional fibrils under certain experimental conditions such as proteolytic treatment and mechanical dispersion[28, 65, 66] (fig. 7). In islets exhibiting a massive proliferation of tight junctions, glucose still elevated insulin release, but large masses of secretory material accumulated in the intercellular space, as if they were trapped in microregions delimited by tight junctional barriers[66]. Glucose further increased tight junction density of enzyme-treated islets[70]. Thus, although tight junctions were so far not associated with the normal insulin release process, it seems quite possible that they regulate pancreatic hormone delivery in pathologic conditions of the pancreatic organ.

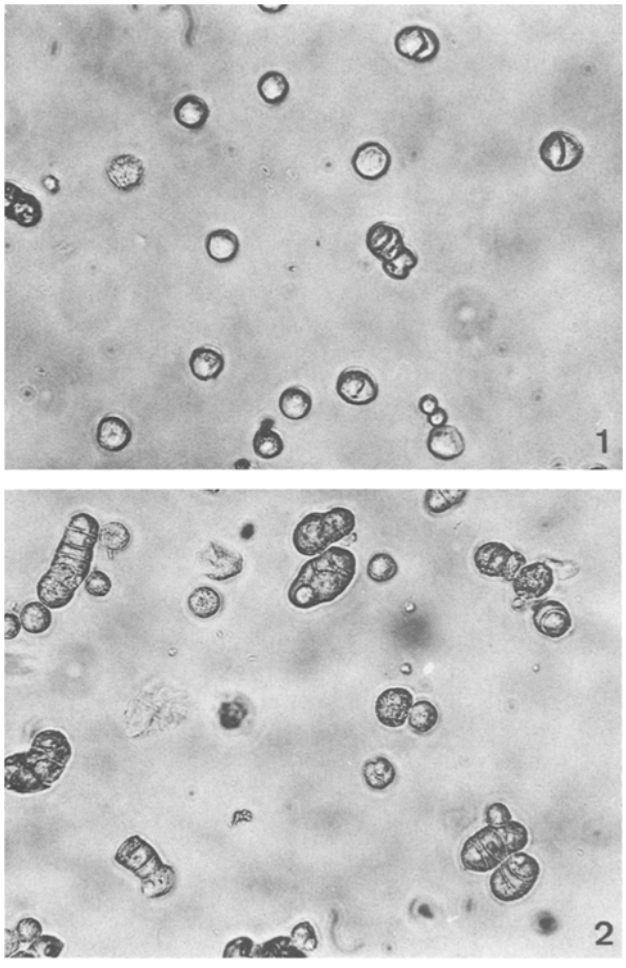

Figure 6. Effect of glucose upon reaggregation of single B-cells. Representative phase contrast photographs after single purified B-cells were shaken for 120 min at 37 °C in the presence of 1.4 mM glucose (1) or 20 mM glucose (2). (Photographs taken during the study reported in Maes and Pipeleers[47]).

3.3 *Gap junctions*

If gap junctions regulate the secretory activity of B-cells, it is likely that this effect will be modulated by alterations in number, distribution and permeability of gap junctions. Experiments wherein such alterations are induced by agents which are known to affect insulin release, are therefore suggestive for a role of gap junctions as regulatory sites in the insulin release process. The number of gap junctions and gap junctional particles has been quantified in freeze-fracture replicas of pancreatic tissue[27], isolated islets[53, 54, 55] and purified B-cells[27]. In all studies, the extent of gap junctions between B-cells varied with the function of these cells. An increase was observed after stimulation by glucose or glibenclamide[27, 53, 54, 55], but also after inhibiting insulin release with diazoxide[58]. While no association could be established between gap junction formation and the secretory activity of B-cells, an inverse relationship was discerned between the surface of gap junctions and the insulin content of B-cells[55]. In analogy to other cell types[12], cyclic AMP was identified as a regulator of the number of gap junctions between pancreatic B-cells at least over a 20-h incubation period[27]. The increased number of gap junctions between islet cells cultured at high cyclic AMP levels[27] explains the increased intercellular communication which has been observed under similar circumstances[39]. An increased formation of structures that permit intercellular communication with B-cells may thus mediate a subacute or chronic regulation of the insulin release process.

Variations in gap junction permeability might transmit acute influences upon insulin release. Such event can be monitored via the flux of ions[60], dye molecules[62], nucleotides[56] or metabolites[38] between neighboring cells. Ionic and metabolic coupling between B-cells has been demonstrated in isolated islets[58, 62] and in islet monolayer cultures[38, 39, 57]. Under basal in vitro conditions, coupling between B-cells is limited to clusters of 2–8 cells[39, 57]. The number of communicating cells can be significantly increased by short exposure to 16.7 mM glucose or to the calcium ionophore A23157[39]; their number rapidly decreased in the presence of somatostatin[39]. As these three experimental conditions are also known to rapidly induce alterations in calcium metabolism in islets, it seems possible that the permeability of gap junctions between B-cells is regulated by the local cytoplasmic calcium concentrations, as has been documented for other cell types[45].

It can be concluded that gap junctions with B-cells can adapt intercellular communication to acute or chronic alterations in the metabolic and hormonal environment. Whether, how and to what degree this membrane process influences insulin release is not yet clarified.

Conclusion

Microscopic explorations of the endocrine pancreas have set the stage for physiologically attractive models on functional cooperation between islet cells. The present review examines the concept that intercellular interactions in islets aid pancreatic B-cells in responding rapidly and appropriately to the metabolic needs.

Indirect intercellular interactions with pancreatic B-cells could occur via locally released cell products such as prostaglandins, opioid peptides, biogenic amines and cyclic nucleotides. Although these substances affect insulin release in vitro, their role as local chemical mediators in islets remains speculative until their interstitial concentration is shown to reach biologically active levels.

Indirect interactions can also be mediated by pancreatic hormones. Conditions were reported where circulating insulin, glucagon and somatostatin altered the secretory activity of B-cells; it is still unknown whether plasma pancreatic hormones regulate insulin release at normal peripheral levels and whether such effects result from their binding to specific receptors upon the pancreatic B-cell. Pancreatic hormones were also considered as possible intra-islet regulators of insulin release, acting via a paracrine route or a short passage in the islet capillary network. In the intact pancreatic organ, an intra-islet effect was documented for somatostatin but not for glucagon. Insufficient insights in the islet interstitium and its irrigation make it impossible to assess the relative impact of paracrine or local endocrine effects in

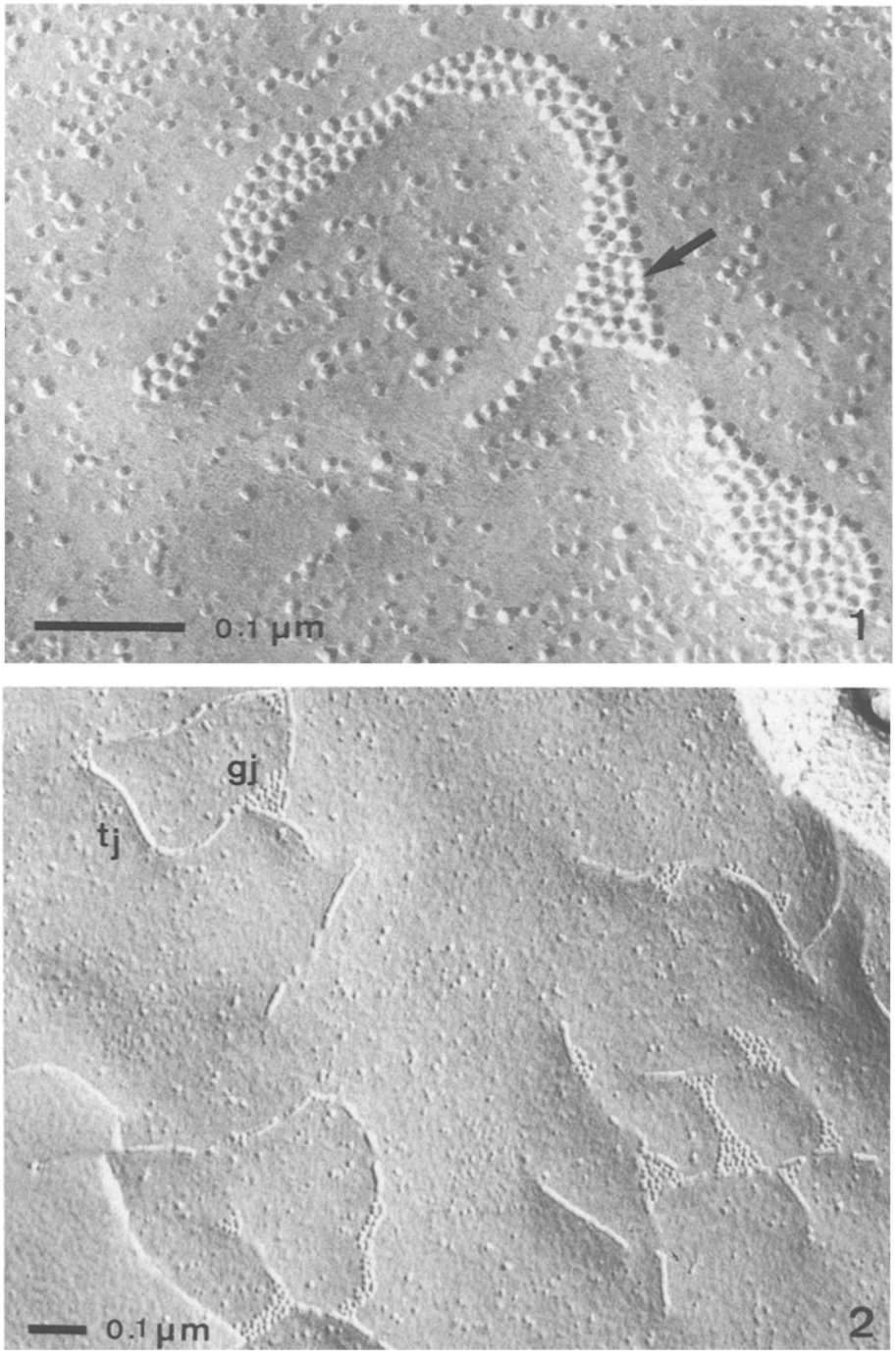

Figure 7. Occurrence of tight junctions in rat islets of Langerhans. Freeze-fracture replicas of rat islets *in situ* displayed gap junctions (arrow) but no tight junctions (1). After their collagenase-isolation, the islets contained tight junctional strands (tj) associated with gap junctions (gj) (2). (Photographs taken during the study of in't Veld et al.[28]).

vivo. In isolated islets, locally released glucagon and somatostatin affect the secretory activity of B-cells. In the absence of these hormones, glucose-stimulated purified B-cells release markedly less hormone than in the presence of non-B-cells or their secretory products. It is suggested that endogenous glucagon maintains the B-cell cyclic AMP levels in islets at levels which permit an immediate hormone discharge upon a sudden rise in glucose; part of the islet B-cells might escape this mechanism as they are closely associated to somatostatin-containing D-cells. Further work on purified islet cells is necessary to confirm this concept, to identify and characterize pancreatic hormone receptors upon islet cells and to evaluate their regulatory role in insulin release.

Islet cells also interact directly with pancreatic B-cells. Experiments on single and reaggregated islet cells indicated that insulin release from intact islets is in part determined by islet cell contacts. Various mechanisms can account for this phenomenon, such as a membrane-induced cell polarity or an exchange of signal molecules through gap junctions. Tight junctions were so far not associated with the normal insulin release process, but might regulate pancreatic hormone delivery in pathologic conditions.

It is concluded that the insulin release characteristics of

isolated islets are determined by both direct and indirect islet cell interactions with pancreatic B-cells. Whether the B-cell dependency on extracellular factors is also met in vivo by locally released hormones and by islet cell contacts is not yet clarified. A possible regulatory role of somatostatin-containing D-cells has been documented in both normo- and hyperinsulinemic conditions. It is conceivable that insulin release in vivo is modulated by islet cell contacts, since membrane interactions with B-cells were described as dynamic events which adapt to acute or chronic alterations in the metabolic and hormonal environment.

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Interaction of sulfonylurea with the pancreatic B-cell

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Key words: Pancreatic B-cell; interaction of sulfonylurea.

Introduction

Although hypoglycemic sulfonylureas may have several effects which are beneficial for the diabetic patient, there is no doubt that their ability to stimulate insulin release is an essential property[49, 64]. This insulinotropic capacity has previously been reviewed[32, 44, 56, 63] and is discussed in this article with emphasis on such mechanisms of action as are thought to be shared by various sulfonylurea derivatives with vastly different potencies. Attention is paid to how the insulin-releasing actions relate to the binding of sulfonylureas to B-cells and to the ensuing effects on metabolism and ion fluxes in these cells.

General aspects of the effects of sulfonylureas and related analogues

As covered in more detail by previous reviews, both first and second generation sulfonylureas can stimulate insulin release in the absence of glucose but are more effective as potentiators of glucose-initiated secretion. The detailed dynamics of the secretory response differs between various drugs, but rapidity of onset is a general characteristic. The immediate response of the B-cell to an acute challenge with sulfonylurea is significantly faster than the response to a sudden increase of glucose from basal to stimulatory concentrations. This difference is a reason for assuming that sulfonylureas act on a distal sequence of events in the physiological signal chain in the B-cell.

Cyclic AMP is in general an intracellular messenger effecting potentiation of insulin secretion in the presence of some initiator. The fact that sulfonylureas can raise the islet cyclic AMP level may therefore contribute to their potentiating action[21]. Whether the effect on cyclic AMP is due to the phosphodiesterase inhibiting properties of sulfonylureas is questionable in the light of data suggesting a poor ability of the drugs to enter into the B-cells (see below). There is an intricate inter-