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Secretory granules

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Introduction

The pancreatic B-cell in common with many exocrine and endocrine cells stores its secretory product in membrane-limited intracellular vesicles and releases it in a graded response through regulation of membrane fusion events. By releasing the hormone from preformed stores a more rapid and sustained response may be achieved than could be attained by control exerted at the translational or transcriptional level. Such a process, however, does require the coordinated synthesis and precise segregation of a large number of proteins and lipids which are specifically required for the vesicle formation and function.

The secreted product is itself of heterogeneous composition. The granule matrix of the insulin secretory granule not only contains insulin and the related peptides, proinsulin and C-peptide, but also a number of other peptides among which are found the proteases required to process the prohormone.

The movement of the granule within the cell, its interaction with other cytoskeletal elements and the fusion reactions of its membranes are likely to be mediated by proteins on the external surface of the granule membrane. These might, either directly or indirectly, respond to changes in the concentrations of second messengers generated by secretagogue stimulation of the cell. A further level of complexity which will be reflected in the granule protein composition is that the granule itself participates in regulation of the concentrations of these same second messengers.

The objective of this review is to consider current knowledge of the nature and functions of the proteins found in isolated insulin secretory granules. To a large extent the physical properties of the organelle and its interaction with other elements of the cell are not considered. The reader is referred to other articles in this series for these aspects and to previously published reviews on this subject[9, 27, 30, 43].

Experimental models

A major obstacle to the chemical analysis and investigation of the biochemical properties of the secretory granule of the pancreatic B-cell is the limited amount of material provided by mammalian islets and the cellular heterogeneity of the material so obtained. A number of recent studies including our own have therefore been performed with B-cell tumors and derived permanent cell lines. The transplantable rat insulinoma used in this laboratory[12] retains a high insulin content and a relatively normal morphology and has been useful for the preparation of subcellular organelles and purification of cellular proteins. The tissue yield per animal is approxi-

mately 2000-fold that using islets as a source. A derived permanent cell line[23] has similar secretory properties to the solid tumors[48, 68, 76] but a much lower insulin content. Both tissues appear to release insulin by an exocytotic mechanism similar to that described in islets. The insulinotropic response to glucose however is normally very limited, a factor which may relate to the reduced activity of the glycolytic enzyme, glucokinase, in the tissue[25, 52]. Glucose sensitivity, however, may be restored upon transplantation into an appropriate tissue site[26]. The solid tumor tissue can synthesize and process proinsulin at a similar rate to that in islets (K. Docherty and J.C. Hutton, unpublished findings) and store the product in secretory granules of a similar morphology to those in islets, albeit of a somewhat reduced diameter[12]. Such tissue has proved a highly-suitable model for studying the molecular events of secretion. Nevertheless it should be remembered that transformed cells frequently synthesize and secrete novel gene products[73].

Secretory granule isolation

Methods which have been developed for the isolation of insulin secretory granules have invariably used as an initial step, the homogenization of tissue in isotonic media of low ionic strength and acidic pH followed by differential centrifugation[9, 10, 31, 40, 45, 77]. The crude granule fraction so prepared contains a mixture of granules, lysosomes, microsomal membranes together with a proportion of the tissue mitochondria. For many experimental purposes further purification is unnecessary, for example, when a granule-specific property is under investigation. However, where activities such as proteolysis, Ca^{2+} transport or ligand-receptor interactions are considered contamination with other organelles can seriously impede interpretation of the results obtained. Further purification has been based principally on density gradient centrifugation techniques. Other methods such as filtration[28] and polymer phase separation[10] have not been widely used. Sucrose density gradient centrifugation methods[10, 28, 40, 50, 77] yield granule preparations with a high insulin specific activity (typically 10–12 U/mg protein) but which are still contaminated with lysosomes and mitochondria. Methods using isoosmotic media based on colloidal silica (Percoll)[35] or metrizamide[50] produce granules with a similar insulin content but with a lower contamination of these other organelles. Such granules have a diameter and morphology similar to that seen in electron micrographs of the parent tissue. A further important advantage of the isoosmotic techniques appears to be an increase in the stability of the isolated organelle. The hyperosmolarity encountered by granules on sucrose density gradients appears to damage the granule membrane and a significant proportion of the soluble granule matrix may be lost[50]. Upon reconstitution in isoosmotic buffer it is probable that further osmotic damage results. Thus granules prepared by sucrose density gradient methods have a higher spontaneous release of insulin than granules isolated in isoosmotic Percoll media[10, 35]. They are not lysed by further reduction in osmolarity and their solubility properties are reminiscent of Zn^{2+} · insulin crystals. Such granules may thus lack a functional lipid envelope.

The granule matrix proteins

Given that the specific activity of insulin in isolated granules (approximately 12 U/mg protein) is a true reflection of that of granules in vivo and that an equimolar concentration of C-peptide is present, it may be calculated that the insulin-related peptides constitute approximately 75% of the total granule protein. The granule membrane protein has been estimated as being 17% of the total[36] leaving 8% to be accounted for amongst other matrix protein constituents. The intragranular water space determined in isolated insulinoma granules approximates 2 µl/mg protein[32], thus insulin content will be around 24% (w/v), C-peptide 14% (w/v) and proinsulin 1.2% (w/v). Of the three peptides only the last is likely to be in a soluble state and even then be present as higher order aggregates.

Electrophoretic analyses of the granule matrix peptides extracted into dilute alkaline media confirms the preponderance of insulin related peptides in the matrix. It reveals as well some 25 other peptides[35]. Two of these, of M_r 5000 and 7000 daltons, are identifiable as intermediates in the processing of proinsulin to insulin on the basis of pulse-chase experiments and their immunoreactivity on gel replicas (unpublished findings). The biological properties of the remainder, however, remain to be established. Prominent among these are a series of proteins of 5000–15,000 M_r, and proteins of 21,000, 26,000, 28,000, 31,000 and 60,000 M_r.

Experiments in which B-cells of tumor origin were prelabelled with radioactive amino acids and then stimulated to secrete have shown that at least 12 of these matrix proteins are co-secreted with insulin[75]. The dependency of their secretion on secretagogue concentration and release with different secretagogues parallels that of insulin which supports the view that they are stored within the same organelle. Seven of the co-secreted peptides of tumor cells coincide with peptides released from isolated rat islets stimulated with glucose, further suggesting a qualitative similarity between the composition of the insulinoma granule matrix and that of the normal B-cell.

Recent studies in a variety of endocrine and neurocrine tissues suggest that many secretory tissues store and release a range of hormone-like molecules besides their principal secretory product[44]. The pancreatic islet is no exception in this regard; A-cells and D-cells contain B-endorphin immunoreactive substances[24, 87] and the B-cell prolactin-like[54] and TRH-like material[38]. The prolactin immunoreactivity has been localized to the cytosol. The TRH-like material, however, may be in the granule since it is synthesized de novo in the islets[56] and released in a regulated manner[17]. The physiological importance of such hormones to islet function remain to be established.

Proteinases

Among the insulin granule matrix peptides may occur the enzymes which are responsible for the protoeolytic conversion of proinsulin to insulin (for reviews see

Chance[11] and Docherty and Steiner[16]). A greater or lesser proportion of the enzymes may also occur in association with the granule membrane[20,79].

Pulse-chase experiments suggest that proinsulin conversion may commence within the Golgi apparatus and certainly continues after granule formation, the half time of conversion being approximately 60 min[16,20,78,79]. Two catalytic activities are postulated to be involved in this process, an endopeptidase which cleaves the proinsulin molecule on the C-terminal side of the two sites in the molecule containing dibasic amino acid residues, and a carboxypeptidase which subsequently removes the C-terminal basic residues to finally generate native insulin, C-peptide and free arginine and lysine molecules. Such a mechanism is inferred from consideration of the structures of the reactants and the experimental observation that a combination of trypsin and carboxypeptidase B from the exocrine pancreas can catalyze the overall reaction[39]. Neither of these enzymes, however, are thought to be responsible for proinsulin processing in the granule. Trypsin recognizes peptide sequences having single basic amino acids and thus also cleaves the insulin B chain. The fact that a proinsulin variant containing a single basic residue rather than a dibasic sequence at the C-peptide/A chain junction is not properly processed[70], also suggests that the endopeptidase recognizes the dibasic sequence specifically. Other proteinases which, like trypsin, have an active serine residue at their catalytic site have been proposed as the endopeptidase, for example, a form of glandular kallikrein[59] or plasminogen plus plasminogen activator[86]. The former enzyme, which is a member of the broad class of enzyme activities implicated in propeptide processing in exocrine tissues[49] has been localized to the pancreatic B-cell, although not specifically to the secretory pathway[58]. A related enzyme isolated from pancreas was found to use proinsulin as a substrate, however, the resultant peptide products have not been unequivocally identified as normal processing intermediates[59]. Plasminogen activators of M_r 44,000, 70,000 and 74,000 have been identified in islets and appear to be localized in the granule since enzymic activity was co-secreted with insulin. The combination of human urokinase and plasminogen obtained from other sources was shown to convert proinsulin to a peptide which had an identical electrophoretic mobility to insulin. Furthermore, this combination of enzymes did not to degrade insulin[86]. A difficulty in the proposal of the serine proteinases as processing enzymes is their generally alkaline pH optima and the fact that their activities are inhibited by diisopropylfluorophosphate and phenylmethylsulphonylfluoride, neither of which inhibit processing in intact or lysed granule preparations[13,20].

Inhibitor studies rather point to the involvement of another class of proteins, the thiol proteinases which with their acidic pH optima appear more suited to the intragranular environment. An enzyme of this type has been implicated in proinsulin, proglucagon and prosomatostatin processing in anglerfish islets[20], in proinsulin processing in rat islets[13] and more generally in propolypeptide processing at dibasic amino acid residues in other tissues[64]. Two proteins of the this type, of molecular sizes of 31,500 and 39,000 daltons, have been demonstrated using ^{125}I-labelled peptide inhibitor affinity probes in pancreatic islets[13] and in lysosomal and secretory granule fractions prepared from a transplantable rat insulinoma[15]. Both peptides can be released by osmotic lysis of the granule but neither appear to be major protein constituents of the granule. The 31,500 M_r protein is similar in size and immunological properties to the lysosomal enzyme cathepsin B. The higher M_r protein, which is in a relatively greater amount in the secretory granule than in lysosomes, appears to be a related enzyme since it can be converted by treatment with pepsin to a form similar to the 31,000 M_r protein. It has yet to be established whether the immunological similarities to cathepsin B extend to their catalytic properties or whether they are active in processing proinsulin. Cathepsin B itself although expressing a high affinity towards synthetic peptide substrates with dibasic amino acid sequences does not correctly process proinsulin, or for that matter, other hormone related peptides[3,55].

Carboxypeptidase activity has been demonstrated in crude preparations of insulin secretory granules[40] and has been further characterized using secretory granule preparations from a rat insulinoma[14]. This enzyme, unlike exocrine pancreatic carboxypeptidase, has an acidic pH optimum and is stimulated by certain divalent cations, notably Co^{2+}. In this respect it is similar to an enzyme termed enkephalin convertase in chromaffin granules of the adrenal medulla[22]. The insulinoma carboxypeptidase appears to be localized principally to secretory granules and can be released by osmotic lysis. Comparison of the specific activity of purified insulin granule carboxypeptidase to the activity in the granule indicates that the enzyme constitutes 1–2% of the granule matrix protein. It is a glycoprotein, its molecular size on gel filtration of 54,000 daltons approximates its M_r determined by SDS gel electrophoresis suggesting it to be monomeric (H.W. Davidson and J.C. Hutton, unpublished findings). Its activity is specific for synthetic substrates with C-terminal basic amino acids. Its physiological substrates have yet to be determined.

Proteinases other than those involved in prohormone processing are also likely to be present in insulin granules. Dog proinsulin, for example, is cleaved at a single basic amino residue in the C-peptide region[42] and a proportion a human, rat and pork proinsulin is cleaved at a Leu-Ala sequence in their C-peptides, suggestive of the presence of a chymotrypsin-like activity[11]. It is conceivable also that granules contain proteases involved in their senescence or active during the exocytotic event. Given that such a mixture of enzymes exist within the organelle it would appear either that they are highly specific for their substrates or that normally their activities are masked. In the latter context it is important to consider the possible influences of the intragranular environment in these reactions, e.g. the high concentrations of protein, the physical state of the enzymes and substrates and the presence of ions such as Ca^{2+}, Mg^{2+} and phosphate at concentrations approximating 100 mmol/l intragranular water[36]. Such factors may, in part, explain the difficulty in demonstrating quantitative proinsulin conversion with exogenous substrates in disrupted granule preparations.

The granule membrane proteins

Insulin granule membranes have been prepared from insulinoma tissue by osmotic lysis at alkaline pH and subsequent treatment with buffers of high ionic strength containing chelating agents. Such preparations contain of the order of 100 different peptides as evaluated by electrophoresis[35]. The molecular sizes of these range from 10,000–100,000 daltons and each are present in similar amounts. At this level each protein would be represented on average by less than 100 copies in each granule.

Direct comparison of electrophoretic analyses of insulin granule membranes to bovine chromaffin granule membranes has not revealed any striking similarities in composition. Immunological staining of electrophoretograms of insulin granules with antisera raised to the bovine chromaffin granule proteins, cytochrome b_{561}, chromogranin and dopamine B-hydroxylase moreover were negative (J. Phillips and J.C. Hutton, unpublished findings). One is left with the impression that the majority of peptides within any one granule membrane type perform specific functions for that particular cell. Similar conclusions have been reached from comparisons of chromaffin and pancreatic zymogen granule membranes[71].

The proton pump

The existence of a proton pump in the insulinoma secretory granule was initially suggested by the finding that osmotically protected freshly prepared material expressed latent Mg ATPase activity which was stimulated by the protonophore FCCP or ionophore combinations which would result in increased proton permeability of the membrane[33]. Such studies moreover defined that the insulin granule membrane was relatively impermeable to H^+, K^+, NH_4^+ and SO_4^{2-} but permeable, in increasing order, to phosphate, acetate, Cl^-, I^- and SCN^-. Like the proton translocating ATPase of mitochondria the granule enzyme, when membrane bound, was inhibited by tributyltin and N,N′-dicyclohexylcarbodiimide. It could be distinguished from the mitochondrial ATPase by its insensitivity to oligomycin, efrapeptin, and azide. Its nucleotide specificity (ATP > GTP > ITP) and cationic requirements (Mg > Mn > Co > Zn = 0) also differed from the mitochondrial enzyme[34].

Radioisotopic distribution experiments with isolated insulinoma granules indicated that nucleotide hydrolysis by the granule ATPase could cause both the acidification of the granule interior and the establishment of a transmembrane electrical potential positive inside relative to the exterior[32,34]. Both chemiosmotic gradients appeared related to a common process, namely the electrogenic translocation of protons across the membrane coupled to ATP hydrolysis on the cytoplasmic surface of the organelle.

The specific activity of the insulin secretory granule Mg ATPase is of a similar magnitude to that reported in the chromaffin granule and the enzyme has similar properties with resepct to its nucleotide and cation specificity and inhibitor sensitivity[2,57]. The subunit composition as revealed by electrophoretic immunoblot procedure using antisera directed against bovine heart F_1 ATPase also showed it to be similar to the chromaffin granules enzyme (J. Phillips and J.C. Hutton, unpublished findings)[2]. More recently the existence of oligomycin-insensitive proton pumps have been inferred in lysosomes[69], coated vesicles and other intracellular organelles associated with the pathway of endocytosis[89]. Some of these enzymes may differ in nucleotide and cation requirements from that of the secretory granules. Nevertheless such enzymes would appear to a general feature of intracellular vesicles which are capable of internal acidification.

Functions of the proton pump

The establishment of chemiosmotic gradients across the granule membrane provides a means of coupling the free energy of ATP hydrolysis on the cytoplasmic face to useful work performed either within the granule or across its membrane. One might expect that it may be coupled to a number of disparate energy-requiring processes by the organelle. These could equally be associated with the establishment of concentration gradients of small molecules across the granule membrane, segregation of granule constituents, morphogenesis or conformational changes occurring upon exocytosis.

The internal pH of freshly isolated granules incubated under iso-osmolar conditions appears to be acidic[1,32]. In the insulinoma granule the internal pH may be up to 2 units below that of the medium. This would indicate that in vivo the intragranular pH may be between 5 and 5.5. The residual acidification of isolated granules appears principally to be the result of activity of the proton pump in vivo prior to isolation. The pH gradient is maintained by virtue of the low permeability of the granule membrane to protons and the high internal buffering capacity of the granule associated with its constituent proteins, phosphate and nucleotides[36]. A Donnan equilibrium generated by the presence of fixed negative charges within the granule would also conceivably contribute to this phenomenon.

Hormone storage

Acidification in the range of pH 5–5.5 corresponds to the isoelectric point of insulin and as such will affect the solubility of the hormone and its crystallization[82,83]. Maintenance of the pH within this range may be critical to the formation of Zn^{2+}·(pro)insulin hexamers since it approximates the apparent acid dissociation constant of the histidine residues responsible for Zn^{2+} binding[82]. As mentioned previously, putative enzymes of proinsulin processing show sharp pH optima in this range[13,14,20].

Amine storage

The transmembrane pH gradient appears to be the principal factor responsible for the concentration of biogenic amines in the insulin granule. Considerable species variation is evident in vivo in the form and extent of accumulation[18,62]. This may in part be due to the availability of the amines or their precursors in the cytoplasmic compartment since pharmacological ma-

nipulations on the animal can lead to increases in islet content of several hundred-fold over the normal content[5]. Isolated granules can accumulate 5-hydroxytryptamine to concentrations 20-fold higher than that of the extragranular medium by a simple diffusion process and can achieve internal concentration as high as 100 mmol/l intragranular water[37]. Such accumulation occurs principally as a consequence of permeation of the membrane by the free base followed by its protonation in the acidic interior of the organelle. The ratio of the intragranular to extragranular amine thus mimicks the proton gradient. It is a passive process and affected by ATP only in the circumstances where ATP hydrolysis is manipulated to cause changes in the pH gradient across the granule membrane. Such a mechanism differes from that responsible for the active accumulation of amines in classical amine storage granules[57]. In this instance the granule membrane has a specific amine transporter which can exchange the amine in the cationic form with two protons translocated by the ATPase. Amine uptake is thus actively driven and dependent both on the pH and electrical gradient established by the proton pump. Such a process allow the establishment of gradients of several thousand-fold and is distinguished from the passive transport mechanism by its dependence on ATP and its inhibition by reserpine. It would appear that such a transporter is at best a minor active constituent of the insulin secretory granule.

Exocytosis

An indication that the chemiosmotic properties of the insulin granule may be involved in exocytosis comes from the inhibitory effects that a number of amines have upon insulin secretion. Compounds such as NH_4^+[72], organic primary amines[8] and imidazole[47] when used at millimolar concentrations all share this property. This is not to deny that each may have other targets within the B-cell and effects on cytoplasmic pH. However, they may all be expected to collapse the pH gradient across the granule membrane, enhance the membrane potential established in the presence of ATP and, if a permeant anion is present, to cause granular swelling. The reported effects of benzylamine on granule morphology support this prediction[63]. In the case of NH_4^+, exposure to 2.5 mM NH_4^+ extracellularly results in the nett intracellular concentration of 40–50 mmol/l intracellular water. The intragranular concentration under these conditions could well exceed 200 mM; a level sufficient to overcome the buffering capacity of the organelle[37].

A hypothetical model has been proposed in the case of the adrenal chromaffin granule of how its chemiosmotic properties may be involved in membrane fusion/fission events occurring during exocytosis. It is envisaged that as the granule becomes exposed to the higher extracellular Cl^- concentration that the ion will permeate the granule membrane in response to the membrane potential generated by ATP hydrolysis leading to the osmotic accumulation of HCl. Subsequent osmotic swelling is viewed as a necessary step in the expulsion of the granule contents to the external medium. A prediction which can be made from the proposed mechanism is that secretion from intact cells will be blocked by raising the extracellular osmotic pressure, replacement of Cl^- in the media by an impermeant anion, by inhibition of the anion transporter or collapse of the chemiosmotic gradients with a protonophore. This is the case both for catecholamine secretion by adrenal cells[66, 67] and insulin release from isolated pancreatic islets and perfused pancreas[74]. When such investigations have been extended to consider different permeant anions, however, a poor correlation between the effects on the chemiosmotic properties of the granule and the anionic requirements for secretion is evident[66]. Also the anion transport inhibitors based on stilbene disulphonate while affecting granule lysis are not inhibitory to secretion. It may be then that the plasma membrane or the granule/plasma membrane hybrid produced at exocytosis is the functional chemiosmotic site. Since islets permeabilized by high voltage discharge still secrete it can be inferred that the plasma membrane per se is not involved in these phenomena[90]. The anionic requirements for secretion in permeabilized islets, however, appear not to coincide either with that of intact cells or to the permeability characteristics of isolated granules.

The mechanism proposed by Pollard and colleagues assigns an essentially permissive role to the proton pump in secretion. However, it may be that the enzyme is regulated in vivo. Glucose stimulation of cultured islet cells has been reported to result in the intracellular accumulation of the permeant fluorescent base 9-aminoacridine presumably as the consequence of granule acidification[63]. The experimental conditions used by these authors do not allow the distinction between the effect of glucose to restore cellular ATP and its potential as a secretagogue.

Protein kinases and their substrates

Covalent modification of proteins by phosphorylation constitutes an important mechanism for the control of their catalytic and biophysical properties and is of widespread importance in the regulation of cellular events including secretion. Regulation of the activities of proteins associated with the insulin granule membranes by phosphorylation conceivably provide a means of controlling protein interactions which may be the basis of granule movement or the recognition of exocytotic sites. Isolated insulinoma secretory granules incubated in the presence of ^{32}P labelled ATP show little protein phosphorylation either alone or in the presence of the putative second messengers Ca^{2+}, cyclic AMP or cyclic GMP[6]. However a number of Ca^{2+}-dependent phosphorylation reactions involving the granule membrane are revealed upon combination of such granules with cytosolic proteins. Three proteins of 10,000, 29,000 and 100,000 M_r are phosphorylated in a reversible manner in response to free Ca^{2+} concentrations in the micromolar range. The first two reactions appear specific to the secretory granule. The 10,000 M_r protein has the interesting property that, once phosphorylated it dissociates from the granule. The reaction involving the 29,000 M_r granule membrane protein results from the activity of a Ca^{2+} and phospholipid-sensitive protein kinase (protein kinase C)[7], an enzyme which has been implicated in the secretory response of islets[84]. The enzyme

has recently been characterized in a purified form from cultured insulinoma cells[46].

In the presence of Ca^{2+}, rat insulinoma granules selectively and quantitatively bind protein kinase C from cytosolic proteins and release it again upon lowering of the Ca^{2+} concentration[7]. This may occur by virtue of the granule membrane providing a lipid matrix since Ca^{2+}-dependent binding occurs to other membranes of insulinoma cells and the enzyme shows Ca^{2+}-dependent interaction with synthetic phosphatidylserine liposomes. Whether the enzyme can be an extrinsic granule membrane protein in vivo, however, remains to be established. Its distribution within the cell may be affected not only by the prevailing Ca^{2+} concentration and relative accessibility of various membranes but also by the membrane lipid composition, in particular to the content of diacylglycerol[41]. Membranar diacylglycerol in turn may be influenced by the turnover of membrane phospholipids, especially phosphatidylinositol and the related polyphosphoinositides. Changes in (poly)phosphoinositide metabolism are usually conceived of as occurring at the level of the plasma membrane in response to receptor-mediated events[19]. However, at least one of the enzymes involved, a phosphatidylinositol kinase is present in the insulin secretory granule membrane[85] and also found in other secretory granule membranes[65]. The reader is referred to other reviews in this series for a more detailed consideration of phosphoinositide metabolism and protein kinase C to the secretory response.

Other proteins

Besides the proteins considered above a long list may be appended of other proteins or activities for which assignment to the granule is less certain. These include intrinsic membrane proteins such as an adenine nucleotide transporter[81], Ca^{2+}-stimulated ATPase[21], 5'nucleotidase[85], a NADPH binding site[88], and a somatostatin receptor[53], and extrinsic ones such as tubulin[80], actin and myosin[4, 29]. Some of the assignments have been made on indirect evidence or using partially purified subcellular fractions. Since in all cases similar proteins occur in other cellular fractions from which contamination has not been discounted their status remains uncertain. One can also add to the list of granule proteins, molecules such as clathrin[60], acid phosphatase[61] and aryl sulphatase[51] which may be associated with the granule transiently during its morphogenesis or senescence.

Conclusions

Although considerable knowledge has been acquired concerning the chemical nature of insulin and its pathway of biosynthesis, relatively little is known about the other proteins which constitute the insulin secretory granule. This, in part, has been a consequence of the difficulties of working with pancreatic islets as a tissue source and the attendant problems of obtaining sufficient material of suitable purity for experimental purposes. The scope for further investigation is perhaps best illustrated by the fact that of the 150 or so insulin granule proteins which may be visualized by electrophoresis the identity of less than 10 has been established. It is clear that some of the proteins will be shared by other granule types in accordance with the common cellular pathways involved in granule morphogenesis and exocytotic secretion. Equally though, it is also apparent that many of the peptides of the insulin granule are tailored for specific functions within the B-cell.

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Insulin secretion: the effector system

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Key words. Insulin secretion, regulation of; microtubule-granule interactions; B-cell cytoskeleton; exocytosis.

Introduction

The vast majority of the chapters in this review deal with aspects of the regulation of the insulin secretory process. They include the recognition of the stimulus, and its translation by means of alterations in ion fluxes, of metabolism, of cyclic AMP generation, of protein phosphorylation and of changes in cytosolic calcium concentrations, into a signal or signals that can be recognized as a final trigger for the mechanical process of insulin secretion. It will be clear from the articles which cover these topics that a huge volume of data is now available which deals with all of these biochemical and biophysical aspects of B-cell function. Much less information is available about the molecular processes which are involved in the mechanism of insulin secretion. In particular little is known of the relationship between the elevation of cytosolic calcium concentrations, which is considered by many to be the final regulator of insulin secretion, and the secretory mechanism itself. We consider here the effector system itself and the way in which its activity might be regulated to increase or decrease rates of insulin secretion.

The cellular events

The insulin storage granules provide a considerable intracellular reservoir of hormone – consisting of some 13,000 granules in each cell[3], of which at least 10% may be secreted in each hour during active periods of secretion. The mechanism of secretion is the transport of granules to the plasma membrane with the subsequent fusion of granule and plasma membranes and release of the granule contents. This latter process is called exocy-