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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-

tosis. The release of insulin and connecting peptide (derived during conversion of proinsulin to insulin) in equimolar amounts[39], together with morphological evidence of correlation of exocytotic events with rates of secretion[16] both infer that this is likely to be the major (and probably the only) secretory mechanism.

Secretion may be dissected, at least in principle, into two distinct events: a) the intracellular movement of granules from the cytoplasmic pool to a position close to the plasma membrane, b) the final release process. Frequent observations of granules aligned along the inner face of the plasma membrane without actually fusing with it attest to the idea that transport of the granules close to the plasma membrane is not in itself sufficient to elicit their final release. We shall therefore consider, firstly, intracellular granule movement and its regulation and, secondly, the final process of granule/membrane fusion.

Intracellular granule movement

a) The role of microtubules

Microtubules were first implicated in the mechanism of insulin secretion by evidence that colchicine[18], and then subsequently a number of other drugs which interfere with microtubule function[11, 19, 43], would all lead to inhibition of insulin secretion. In most cases first and second phases of secretion in response to glucose and other stimuli were affected although colchicine was found to influence only the second phase of glucose-stimulated secretion[19]. Interestingly the nature of the chemical action of the drug on microtubules does not alter its effect on secretion. Thus colchicine disaggregates microtubules by binding to their tubulin subunits (table), shifting the equilibrium between subunits and microtubules in favor of the subunits. Vinblastine causes formation of paracrystals of microtubular protein, while nocodazole induces microtubule disaggregation into their constituent subunits. Conversely, deuterium oxide stabilizes microtubules in a polymerized state, while taxol increases microtubule polymerization (table). Therefore those agents which increase microtubule polymerization as well as those which cause their depolymerization will all produce a similar effect – the inhibition of glucose stimulated insulin secretion. This implies that it is the dynamic turnover of tubulin to microtubules which is important for the mechanism of secretion rather than the total number of microtubules which are present in the B-cell at any moment.

This was confirmed by experiments in which the proportion of the total tubulin content of the islet which was present as microtubules was estimated by binding of ^{3}H colchicine to the subunits in conditions in which microtubules are stabilized. These showed that the proportion of subunits decreased in conditions in which insulin secretion was stimulated[24]. In particular agents which increased cyclic AMP concentrations in islets (theophylline or isobutyl-methyl xanthine) would increase the rates of secretion and increase microtubule polymerization in parallel[24]. This may be a result of increased phosphorylation of microtubule subunits themselves, as has been demonstrated recently in islet extracts by Colca et al.[2], or of phosphorylation of microtubule associated proteins, which has been observed in a number of other systems. Biosynthesis of tubulin which is stimulated by 20 mM glucose has been demonstrated in isolated islets, although no net change in total tubulin content of the cells was observed in short term incubations.

The general question arises as to whether the effects of the antimitotic drugs on insulin secretion are specifically on microtubules themselves, and if so whether the effects reflect a role in the intracellular granule transport mechanism specifically, or merely a disruption of the cytoskeleton which might be expected to interfere with various cell functions. The first of these questions may be satisfactorily answered by reference to the wide variety of drugs with varying mechanisms of action (table). Since each of the agents listed acts by apparently quite distinct chemical mechanisms, it seems very unlikely that their common property is other than an interaction with microtubules. Various studies have also been performed to investigate the specificity of the effects of colchicine and vinblastine in particular on insulin biosynthesis, and 45-calcium handling[22]. In general, they have shown that, when used at the appropriate concentrations, the effects of these agents are relatively specific for the mechanism of insulin secretion. The second question is less easy to answer satisfactorily except to note that there is ultrastructural evidence of the endogenous association of insulin storage granules and microtubules in vivo and that this evidence is most easily seen in monolayer cultured cells where long lengths of microtubules are relatively flattened into two dimensions. Evidence obtained in vitro of the association of granules with microtubules and microfilaments (see below) further supports this hypothesis.

There is also evidence for a role of microtubules in the intracellular transport of microvesicles from the rough surfaced endoplasmic reticulum of the B-cells to the Golgi complex[23], and blockage of this transport step

Compounds which have been used to study the role of microtubules and microfilaments in insulin secretion

	Mechanisms of action	Effects on secretion
Microtubules:		
Colchicine	Inhibits addition of tubulin molecules to microtubules, resulting in depolymerization	Inhibition
Vinblastine	Causes formation of paracrystalline aggregates of tubulin	Inhibition
Nocodazole	Induces microtubule depolymerization	Inhibition
Taxol	Causes tubulin to assemble into microtubules	Inhibition
Deuterium oxide	Stablizes polymerized microtubules	Inhibition
Microfilaments:		
Cytochalasin B	Binds to actin filaments, preventing addition of further actin	Stimulation
Phalloidin	Stabilizes actin filaments	Stimulation (in permeabilized cells)

may be expected to lead to inhibition of proinsulin to insulin conversion[23].

The localization of microtubules in the B-cells with an anti-tubulin antibody has been studied by Boyd et al.[1] in monolayer cultures of B-cells grown and flattened on a coverslip. This allows a great increase in surface area of the cells available for localization of antigens, since the cells illustrated by Boyd et al. are ~ 20 µm in diameter whereas a B-cell in vivo would be expected to have a diameter of 10–12 µm. This raises the question of whether the microtubules observed represent stress fibers induced by culture rather than the configuration of microtubules in the cells. In either case the authors demonstrated convincingly a radiating network of microtubules originating from the perinuclear region which was disrupted by prior exposure of the cells to colchicine (10^{-6} M) but not to the inactive analogue lumicolchicine[1]. The absence of shape change following exposure to these agents may suggest that these microtubules are not solely involved in the maintenance of cell shape, but may rather be specifically involved in intracellular transport processes.

In a recent study in which rats were treated in vivo with low concentrations of vincristine before estimation of microtubule number in B-cells and their insulin secretory responses to glucose and arginine, it was shown that vincristine treatment inhibited glucose-induced (but not arginine-induced) insulin release, in the absence of changes in microtubule number or length within the B-cell[36]. These findings might be explicable in part by the possibility that the lower concentration of vincristine to which the islets were exposed might affect microtubules functionally but not morphologically, although this would not explain the absence of effect on arginine-induced secretion, which earlier in vitro studies had shown to be vinblastine-sensitive in the same way as glucose.

b) Microtubule-granule interactions

The binding of insulin storage granules isolated from angler fish endocrine pancreas to microtubules from brain homogenates has been studied by dark field electron microscopy. Granules were associated with assembled microtubules only when the latter were perifused in the presence of microtubule-associated proteins, but not of purified tubulin alone[41]. Granule binding to these microtubules was reduced by the addition of 0.1 mM ATP but not ADP, and enhanced by the addition of cyclic AMP (0.1 mM).

In a rather different approach, Pipeleers demonstrated the binding of I^{125} tubulin to subcellular fractions of rat islets of Langerhans. An increase in this binding in a storage-granule rich fraction was observed in the presence of 1 mmol/l $CaCl_2$[32].

c) Microfilaments

Actin, believed to be the constituent protein of microfilaments, was first localized in B-cells in 1974[6], although microfilament bundles had been observed previously. Evidence for the involvement of microfilaments in the insulin secretory mechanism consists in part in the use of specific drugs which interfere with their formation, although in comparison to microtubule inhibitors the list of agents is very small (table). Cytochalasin B which induces microfilament hypercontraction, or disruption, by inhibiting the addition of actin molecules to existing filaments, was used initially, and shown to elicit enhanced rates of insulin secretion[44]. This was suggested to be a result of re-distribution of the sub-plasma membrane 'cell web' which would allow ready access of the granule to the plasma membrane for its final release. The frequent observation in electron micrographs of unstimulated B-cells of many granules which are closely aligned along the plasma membrane however suggests that alternative or additional explanations of the role of microfilaments and actin might be sought. In particular, while microtubules might provide the stable mechanical structure along which the granules could move in an orientated direction, as knowledge of their biochemistry increased it became clear that the microtubules themselves could not provide the motive force for the movement of the granules through the cell. At the same time interest in the role of microfilaments in the transport of organelles through the cytoplasm of cells, and of shape change by the cells increased and led to re-examination of the original proposal of Lacy et al.[18] that a combined microtubular-microfilamentous system might be the effector unit for the intracellular movement of the granules. Evidence for this proposal includes the suggestion that isolated granules can interact with microfilaments in a simple in vitro system, and the fact that the regulation of microfilament polymerization is governed by similar factors to those which alter rates of insulin secretion (see below).

In addition, the further stimulation of glucose-induced insulin secretion which is seen in the presence of cytochalasin B can be inhibited by vinblastine, suggesting that the presence of both microtubules and microfilaments are required for the mechanism of secretion to be effective.

Very recently a second drug (phalloidin), which is known to interact specifically with actin and induces its complete polymerization, has been tested. While it has no effect on insulin secretion from intact isolated islets, phalloidin will stimulate insulin secretion from islets which have been permeabilized by a high voltage discharge technique[40]. At the same time it has been shown that fluorescently labelled phalloidin will penetrate permeabilized but not intact islet cells[40].

d) Myosin

The light and heavy chains of myosin were first identified in catfish islets, where it constitutes 1% of the total protein[29] and have subsequently been observed in extracts of rat islets. In addition the enzyme which is responsible for myosin light chain phosphorylation, myosin light chain kinase, has been identified in extracts of normal islets[9,20] and of insulin secreting tumors[31]. The enzyme is readily observed to phosphorylate exogenous myosin light chains in broken cell systems. However, there have to date been few convincing demonstrations of the phosphorylation of endogenous myosin light chains on stimulation of secretion in intact cells (see pa-

per by Harrison et al., Experientia *40* (1984) 1075). There may be technical reasons for this – for instance that the changing specific activity of endogenously labelled ^{32}P-ATP within the cells following stimulation of secretion by glucose masks any such effect, or that the phosphorylation in the intact cells is rapidly reversed by active phosphatases; alternatively it may be that myosin light chains do not provide an important endogenous substrate. The idea that such a phosphorylation does take place but is masked for technical reasons is worth pursuing since it provides a direct link between the elevation of intracellular calcium and the activation of the insulin secretory mechanism. Calmodulin is a constituent subunit of myosin light chain kinase and this enzyme is activated by calcium.

e) Intermediate filaments (Vimentin)

A number of other filamentous structures of varying diameter have been identified in mammalian cells, and may be subject to phosphorylation by calcium or cyclic AMP dependent protein kinases. Of particular interest is a report that a cytoskeletal protein isolated from hamster insulinoma cells, whose molecular weight is 60,000, may be phosphorylated in response to depolarization-induced Ca influx[34]. Two-dimensional peptide mapping suggests that one of these cytoskeletal proteins of mol.wt 60,000 daltons might be the intermediate filament protein vimentin[34], and another protein identified in the same system may be keratin[35]. The same authors also showed that cAMP-dependent phosphorylation in hamster insulinoma cells produced a quite distinct pattern of protein phosphorylation in which two cytosolic 16,000-dalton proteins were predominant[34]. Little is known of the existence of intermediate filament proteins in normal islet B-cells; hamster insulinoma cells are of course unresponsive to glucose stimulation of insulin release.

f) Microfilament/granule interactions

The possible interactions of actin or actomyosin filaments with granules have been studied in a simple model system (fig. 1). Briefly, the results of these studies indicated that filamentous (but not globular) actin[13] and actomyosin (but not myosin alone)[14] were capable of retarding granule sedimentation without altering their stability in these conditions. The effects were specific at least in so far as they were not mimicked by other solutions of comparable viscosity or by fibrous collagen. Ultrastructural studies confirmed that the granules were indeed in close contact with actomyosin filaments[14]. A more detailed study of the regulation of this interaction indicated that of the many factors tested (calcium, ATP, cyclic AMP, calmodulin $\pm Ca^{2+}$) in this admittedly rather crude system only ATP was capable of enhancing the interaction of the granules with actomyosin. Similar results had previously been obtained in a study of F-actin-granule interactions[13].

The question of whether the actomyosin filaments exist in the cytoplasm, perhaps in association with microtubules and the granules become attached to them and are moved along following activation of a contractile system analogous to that seen in smooth muscle, or whether perhaps the myosin is a component of the granule membrane (fig. 2), has been discussed[10]. It is clear that at least in model systems very small quantities of myosin (7–25 molecules per sphere of diameter 0.7 μm) would be sufficient to propel a plastic bead along an oriented actin matrix at a rate of 5 μm/sec[37]. The detection of such a very small number of myosin molecules in association with the membranes of insulin storage granules is likely to prove very difficult by biochemical or by immunocytochemical means. One interesting consequence of the interaction of granules with actomyosin in this way is that a gap, equivalent to the width of the head group, would be expected to separate the actomyosin filament from the granule membrane itself.

The movement of granules within intact cells has been examined directly by cinemicrography[17,38], and they have been observed to move in a discontinuous way along directed pathways at speeds of 0.8–1.5 μm/sec. The frequency of these 'saltatory' movements was increased during exposure of the cells to high glucose concentrations which stimulate insulin secretion. Conver-

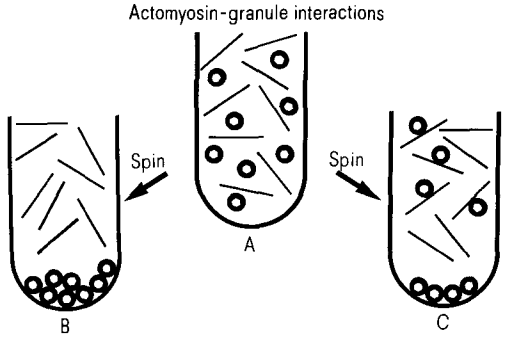

Figure 1. Schematic diagram to show an in vitro system to test the interaction of isolated insulin storage granules with cytoskeletal components. After incubation of the two components at 37 °C (A) the tube is centrifuged. If no interaction of granules with cytoskeletal components occurs, the granules will sediment preferentially (B), leaving a low insulin content of the supernatant. If interactions occur however, the insulin in the supernatant will be increased (C) in proportion to the degree of interaction which is observed.

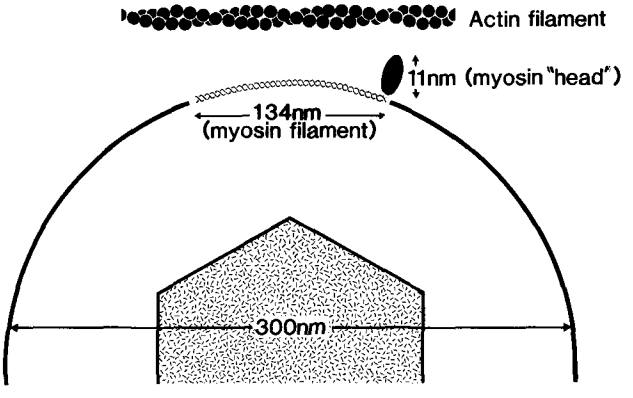

Figure 2. A speculative model showing the way in which myosin, present in low concentrations in the granule membrane, could interact with actin filaments to produce granule movement. Movement of myosin coated plastic beads along an actin pathway has been reported[37]. Reproduced from Howell[10].

sely they were abolished in the absence of extracellular calcium or by the addition of adrenalin, each of which will inhibit secretion. Microtubule disrupting agents (vinblastine, deuterium oxide) inhibited granule movement, while cytochalasin B which induces microfilament hypercontraction affected the cell boundary and either stimulated[17] or had no effect on[38] granule movement. This implies that cytochalasin B might affect microfilaments within the cell cytoplasm as well as in the cell web.

g) The B-cell cytoskeleton

The cytoskeleton of the cell is sometimes considered as the system of microtubules, microfilaments intermediate filaments etc. which is responsible for maintenance of its shape, for any changes of shape and for its motility. This definition clearly envisages a prominent role for the microtubules. Nevertheless, it has recently become commonplace to identify the cytoskeleton operationally as those proteins which remain insoluble after extraction of the cells with Triton X-100 (see e.g. Osborn and Weber[28], and Trotter et al.[42]). The elements remaining after such extraction include microfilaments and a variety of other intermediate filaments but not microtubules which are rendered soluble by Triton X-100 in the conditions used. The cell nuclei may also be insoluble[28]. Some caution is therefore needed in the interpretation of enzyme or other activities which are apparently not associated with Triton-insoluble cytoskeletons, since the cytoskeleton in vivo is likely to represent a rather more complex structure.

h) Microtubule-microfilament interactions

Microtubules and microfilaments play a cooperative role in many cellular functions although their interactions have not so far been studied directly in B-cells. Since it is assumed that the constituent proteins tubulin and actin are common in structure in many cell types, it seems reasonable to suppose that the interactions are governed by the same principles as would apply for tubulin and actin from any other source. Investigations of the in vitro interaction of these two have been reported, and it appears that microtubules and microfilaments do interact in vitro but only in the presence of microtubule associated proteins (MAP's) which may mediate these relationships[7]. MAP's are essential for tubulin polymerization and may be distributed along the entire surface of the microtubules.

Microtubule-actin interactions in vitro can be altered (inhibited) by phosphorylation of these MAP's. The ability of unphosphorylated MAP's to crosslink actin to microtubules is unaffected by pH in the range 6.8–7.4 but falls off rapidly at high pH. The ability of phosphorylated MAP's to crosslink actin is maximal at pH 6.2–6.3[25].

The finding of reduced interaction of actin with microtubules following phosphorylation of MAP's is of particular interest, and if it occurs in vivo may provide a mechanism whereby the 'dynamic' actin component of the microtubular-microfilamentous system could become dissociated from the 'static' microtubule component to facilitate its contraction and the movement of granules. As discussed above, phosphorylation of microtubules or MAP's may be enhanced by the action of cyclic AMP or calcium dependent protein kinases following any stimulation of secretion which raises the intracellular concentrations of either or both of these two mediators.

The activation of the effector system

As discussed in the paper by Prentki and Wollheim (Experientia *40* (1984) 1052) there is now direct evidence using the fluorescent Ca sensitive probe quin-2 that in insulin secreting tumor cells[45] stimulation of secretion is accompanied by an increase in cytosolic calcium concentrations, as well as a wealth of indirect indications that this is the case from studies of calcium fluxes in isolated islets in various conditions. The way in which this increase in calcium can be translated into granule movement and extrusion by exocytosis is therefore of crucial importance.

A number of calcium binding proteins are known which can interact with components of the tubulin/actin/myosin cytoskeleton (see Kakiuchi and Sobue[16]). They include calmodulin which has been identified and characterized in islet cells, which as described above could readily promote myosin light chain phosphorylation, and in turn lead to actin activation and contraction in a system analogous to that which may operate in smooth muscle. Little is known of the presence or role in islet cells of the other actin- and tubulin-binding proteins which are present in various cell types[16], but it is easy to see in a general model how these classes of proteins could act as bridges between calmodulin binding and cytoskeleton activation (fig. 3). There is also evidence for association between cytoplasmic microtubules and calmodulin in 3T3-cells, affording a relationship by which calcium might be able to directly regulate the degree of microtubule polymerization[5]. As is so often the case, the small quantities of pure islet tissue which can be obtained for biochemical studies render any approach to the characterization of calcium and cytoskeletal binding proteins a slow and difficult one.

Nevertheless it is possible to construct at least a speculative model for the way in which microtubules and microfilaments act together to form a functional unit which is responsible for the intracellular movement of

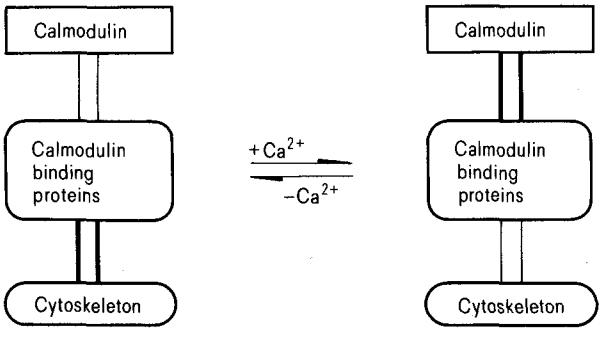

Figure 3. Figure showing the potential importance of calmodulin binding proteins in mediating the effects of activation of calmodulin on the activity of the cytoskeleton. See also Howell[10].

granules, and which at the same time can be regulated by calcium and cyclic AMP, and this is shown in figure 4. The model indicates that increases in calcium and cyclic AMP will enhance formation of microtubules (from tubulin) and F-actin (from G-actin) and that these two together with phosphorylated myosin will comprise the effector system for granule movement[10]. As indicated earlier phosphorylation of the microtubule associated proteins will at the same time dissociate them from intimate contact with the microtubules so that they can act as an effective contractile unit along oriented pathways which are determined by the microtubules.

Granule/membrane fusion

As discussed above, the intracellular movement of granules through the cell may be subject to regulation independent from that of the final process of granule extrusion and thus the granule might initially be transported to a position in relation to the cell membrane which is determined by Van de Waals forces of attraction and by electrostatic repulsion[4]. Dean also considered the possibility that a local increase in calcium concentration under the plasma membrane might neutralize the negative charge on the membranes sufficiently to permit fusion to occur[4]. An attempt to demonstrate the neutralization of negative charges as indicated by the distribution of cationic ferritin[12] in isolated granules by addition of calcium was able to produce a demonstrable change only at rather high concentrations (~ 1 mM) of added calcium. Of course it is not yet possible to determine what concentration of calcium might be reached locally beneath the plasma membrane during a period of enhanced influx following stimulation of secretion.

The chemiosmotic hypothesis of granule release by exocytosis

An additional hypothesis for the facilitation of release of granule contents following contact of the granule membrane with the inner aspect of the plasma membrane was originally advanced by Pollard et al.[33]. It was proposed that the granules swell as a result of chemiosmotic mechanisms which eventually lead to their lysis, resulting in extrusion of the granule contents to the cell exterior. This hypothesis, initially proposed in catecholamine, serotonin and parathyroid hormone secreting cells, was first examined in islet cells by Orci and Malaisse[27] who showed that sucrose and anions such as isethionate were able to inhibit glucose induced insulin secretion, consistent with the existence of a chemiosmotic mechanism of release. Subsequently, Pace and Smith[30] confirmed that hyperosmolar solutions (addition of sucrose), substitution of chloride by isethionate or sulphate would inhibit insulin release, while drugs which prevent anion exchange in erythrocytes (DIDS (di-isothiocyano 2,2′ stilbene disulphonic acid) and probenecid) also inhibited secretion. Replacement of Na^+ by choline did not influence rates of insulin secretion[30]. Problems of the possible indirect effects of some of these ion substitutions and inhibitors were in part overcome by Yaseen et al.[46,47] who used a high voltage discharge technique to render the islets directly permeable to changing concentrations of anions and cations. They concluded that in this more direct system there was no convincing evidence for a role of anions in inducing granule lysis, and considered the possibility, proposed by Grinstein et al.[8], that cations could contribute alternatively in the chemiosmotic process. These experiments are still in progress.

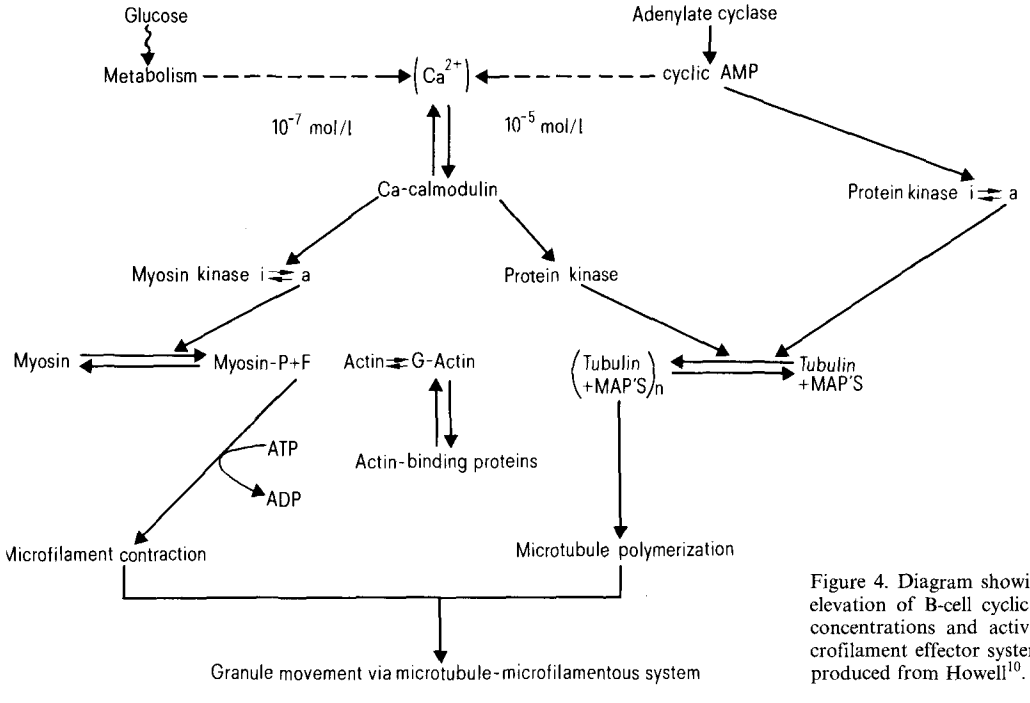

Figure 4. Diagram showing the potential link between elevation of B-cell cyclic AMP and cytosolic calcium concentrations and activation of the microtubule-microfilament effector system for granule movement. Reproduced from Howell[10].

The existence of a Mg^{2+} sensitive ATPase activity has been demonstrated in the membranes of purified insulin secretory granules which were isolated from the cells of a transplantable insulin secreting tumor[15]. It is possible that an increase in granule membrane potential (more positive inside) as a result of activity of the ATPase could in turn promote the transport of anions into the granule interior, resulting in its swelling and eventual bursting. Further studies are needed to evaluate the appropriate role (if any) of such chemiosmotic mechanisms in the insulin release process.

Conclusions

Studies of the role of the microtubule-microfilamentous system in insulin secretion have been widened by continuing experimentation and analysis to provide a comprehensive working hypothesis which embraces ideas of the way in which the polymerization of microtubules and microfilaments may be regulated and how these cytoskeletal components may act together to enhance the process of granule movement. It is also possible to speculate about, but not yet to demonstrate, the way in which the activities of this effector system could be regulated by calcium and by cyclic AMP, which are essentially involved in the regulation of rates of secretion.

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Kinetic aspects of compartmental storage and secretion of insulin and zinc

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Introduction

In the normal animal, the endocrine pancreas stores enough insulin for several hours of stimulated secretion (with a turnover time of approximately 16 h)[82]. In man, with his typical fasting-feeding patterns, stored insulin represents a 5-day supply. Several compounds necessary for the formation, maturation and condensed storage of this hormone are co-stored in secretory vesicles. Some are also co-secreted with insulin and, for a short period of time, may modify the efficacy of insulin at its target organs. Therefore, subtle alterations of storage and secretion of hormone in the B-cell may have profound affects on glucose homeostasis.

Kinetics of insulin secretion

Beta cells, as well as other secretory cells, respond to constant stimulation by secretion of their hormone in multiphasic patterns[94]. Kinetics of these patterns have been studied both to evaluate the underlying biochemical mechanisms regulating minute-to-minute secretion and to establish their possible relevance for total body homeostasis.

Several characteristics of insulin secretion from the perfused rat pancreas are shown in figure 1. When glucose is presented as a rapid onset, constant infusion, there is an immediate burst of insulin for 2–5 min followed by a brief nadir. This is succeeded, in turn, with a second ascending secretion which reaches equilibration with time. Transient negative spikes of insulin release can be produced by suddenly reducing the glucose to a lower stimulating level[49, 94]. Thus, insulin secretion is sensitive to the rate of change as well as the static concentration of a secretagogue.

In addition, prolonged stimulation with glucose produces a time-dependent potentiation of the B-cells, resulting in hypersensitization to a subsequent stimulus (fig. 1). Potentiation is time and dose dependent on the initial glucose stimulation and has a half-time 'memory' of approximately 30 min[16, 46, 47, 51]. Therefore, although the removal of glucose causes an immediate cessation of insulin secretion, metabolic components in the B cell remain, for a limited period, in an activated state. A metabolite of glucose is probably involved since glyceraldehyde is active, whereas non-metabolizable galactose is not[47]. Other fuel substrates (e.g. alpha-ketoisocaproic acid) are effective time-dependent potentiators. Insulin secretion during the priming period is not essential, glucose remaining effective when secretion is blocked by somatostatin and, in some reports, calcium deprivation[47]. The nature of the intracellular factors causing priming are unknown. They probably are similar to those causing the second rising phase of insulin secretion[94], and probably do not involve cyclic-AMP[47]. Other insulin secretagogues such as the depolarizing agents, sulfonylurea or potassium, cause first phase insulin secretion with only a small, sustained second phase; in the presence of low glucose, a more typical multiphasic release results[48, 50, 52, 61].

A number of mathematical models have been described to account for multiphasic insulin secretion (reviewed in Landahl and Grodsky[79] and O'Connor et al.[94]). These models incorporate different structures to provide sensitivity to rate of change and concentration of secretagogue and fall in two general categories. First are 'storage-limited models' in which insulin is presumed to be stored heterogeneously in compartments or pools