Diabetologia (1982) 22: 301–308

Diabetologia

Review Articles

Microtubules, Microfilaments and Insulin Secretion

S. L. Howell and M. Tyhurst

Department of Physiology, Queen Elizabeth College, University of London, London, UK

The first indication that the mechanism of insulin secretion might involve the extrusion of granules by exocytosis came soon after the application of electron microscopic methods to the study of the pancreatic B cell [20]. However, there was controversy as to whether exocytosis was the major mechanism of secretion until studies by freeze fracture provided quantitative evidence that this is indeed the case [37]. Detailed discussion of the mechanism by which granule extrusion is achieved was initiated when Lacy et al. [21] proposed a role for a contractile microtubule/microfilamentous system in the oriented movement of granules (margination) prior to exocytosis. This concept has gained gradual acceptance over the years, and it is the purpose of this brief review to outline some of the information which is now available about microtubules, microfilaments and contractile-system proteins in the pancreatic islets, and their relationship to the processes of insulin secretion.

Microtubules

The experimental basis of the microtubule-microfilamentous hypothesis was the finding that colchicine, an alkaloid known to disrupt microtubules and the mitotic spindle in mammalian cells, was able to inhibit glucose-induced insulin secretion from isolated rat islets of Langerhans [21]. This finding was subsequently confirmed and greatly expanded by studies of the effects of other agents such as vinblastine or deuterium oxide, which are known to disrupt microtubule function,on the dynamic aspects of insulin secretion [22, 50]. Vinblastine and deuterium oxide have been shown to inhibit both first and second phases of insulin secretion while colchicine has generally been found to inhibit only the second phase of glucose-induced secretion. The agents used in these studies have varying mechanisms of action. Colchicine binds to the constituent protein of microtubules (tubulin) and prevents its polymerization into microtubules. Vinblastine, or the related compound vincristine (vinblastine sulphate), causes aggregation of microtubular proteins into large crystalline arrays of protein which are distributed throughout the cytoplasm [28]. In contrast, deuterium oxide stabilizes microtubule structure and increases the number of microtubules which are visible in each cell [28]. Interpretation of the results of such experiments is complicated by the rather long (and uncertain) time periods which are required both for the penetration of the agents into the cells and, once inside the cell, for the agents to exert their effects; some inconsistencies have emerged. In addition, problems of specificity of the drugs need to be considered as there appears to be some evidence that, unless used at carefully selected concentrations, each of these compounds may have effects other than those expected in disrupting microtubules. Thus deuterium oxide and vinblastine have been reported to inhibit ^{3}H-leucine incorporation into islet proteins, although glucose-stimulated insulin biosynthesis was not specifically affected [26], and deuterium oxide has been shown to alter theophylline-induced changes in ^{45}Ca handling by islet cells. Nevertheless, it seems likely that the effect of these agents on insulin secretion can be accounted for predominantly by their actions on microtubular function. It has been suggested [22] that the rapid first phase of secretion might be a consequence of the intracellular transport and extrusion of granules which were already in contact with the microbutules in the resting cells, while the second phase may require the association of granules from the cytoplasmic pool with microtubules before their transport and extrusion is effected in a similar way. This latter could account for the prolonged second phase secretion in response to a glucose stimulus. The

0012-186X/82/0022/0301/$01.60

Table 1. Proteins related to the microtubule and microfilamentous system which have been identified in islets of Langerhans

Protein	Molecular weight (daltons)	Proposed role
Tubulin	55000: dimer of 110000 [34]	Polymerizes to form microtubules
Actin	42000 (globular form, globular-actin)	Polymerizes to form filaments (filamentous actin)
Myosin	460000: composed of two heavy chains of 200000 and two pairs light chains of 14000 and 19000 [39]	ATPase activity enhanced after phosphorylation and by association with actin (actomyosin)
Calmodulin	17000	Calcium dependent regulatory protein

It seems likely that the islets also contain microtubule-associated proteins, actin binding proteins, profilin and probably a range of other related proteins as well.

role of microtubules in the biphasic pattern of insulin release has been reviewed in detail by Malaisse et al. [27].

Effects of colchicine in retarding intracellular transport of proinsulin from rough surfaced endoplasmic reticulum to the Golgi complex, as well as inhibiting secretion of newly synthesized insulin, have also been reported [29]. This inhibition of transfer of newly synthesized material to the Golgi complex was correlated with inhibition of the conversion of proinsulin to insulin. This finding has parallels in the observation of a role of microtubules in the intracellular transport of growth hormone [12] and of pro-parathormone [17] prior to their packaging in storage granules.

Tubulin

In the second stage of the study of the role of microtubules in insulin secretion, attempts were made to analyse quantitatively the amount of microtubular protein (tubulin, Table 1) which was present in the islets in various states of secretory activity. This was made possible by the use of colchicine binding assays in which ^{3}H-colchicine bound to tubulin can be separated from free colchicine by filtration through Whatman DE 81 filters. Initial studies by Montague et al. [34] showed that the colchicine binding protein, which was estimated in this assay, had a molecular weight of 110000 daltons and an intrinsic association constant for colchicine of 1.4 μmol/l, consistent with its identification as tubulin dimer. Uptake of ^{3}H-colchicine into intact islet cells was found to be fairly rapid (30 min), although the association of the drug with tubulin inside the cells was a relatively slow process. The time course of this binding approximately paralleled that of the effects of colchicine on insulin secretion.

Subsequent investigations have compared the equilibrium between polymerized microtubules and their sub-units in different phases of secretory activity, based on the finding that colchicine binds specifically to microtubule sub-units but not to intact microtubules; microtubules in the islets were stabilized by the addition of deuterium oxide to the extraction buffer. Montague et al. [35] showed that stimulation of insulin secretion by glucose or 3-isobutyl-1-methyl xanthine resulted in a decrease in the number of tubulin sub-units which was detectable in the cells as a result of their polymerization, and this was associated with an increased microtubule formation; this polymerization was not observed after incubation in calcium-free medium. In the resting cells, approximately 23% of the microtubule protein was in the form of microtubules, increasing to 36% on stimulation [38]. Dynamic studies of tubulin polymerization by similar methods showed that, during first phase secretion in response to glucose, tubulin polymerization increased from 24 to 33% of the total, falling to basal levels at the end of first phase stimulation, but increasing again during second phase release. However, the increase in polymerization in second phase appeared to be only poorly correlated with the rate of secretion [32]. Cytochalasin B, an agent which interferes with microfilament function (see below), also induced microtubule polymerization in a way which paralleled its effects on insulin secretion [32]. Further, Pipeleers et al. [41] found that starvation of rats produced a reduction of total tubulin in their islets as well as in the proportion of tubulin which was in polymerized microtubules. These changes were reversible on glucose refeeding. These authors identified tubulin by polyacrylamide gel electrophoresis of islet extracts and were able to demonstrate glucose-stimulated tubulin biosynthesis after incubation of islets with ^{3}H labelled amino acids [40]. Finally, the same authors demonstrated binding of ^{125}I-tubulin to various subcellular fractions of rat islets. Of particular interest was the increase in the binding which could be induced by addition of 1 mmol/l calcium chloride, suggesting a possible role for calcium in facilitating microtubule-granule interactions [42].

Two interesting controversies which remain involve the roles of cyclic AMP and calcium in the regulation of microtubule polymerization and, although neither has been studied in islet tissue, it may be useful to review the position as it appears in other cell types. Despite earlier reports to the contrary, it is now clear that tubulin itself has no intrinsic protein kinase

activity, the endogenous kinase activity being separable from tubulin by column chromatography [7]. The substrate for this cyclic AMP dependent kinase appears, in some tissues, to be a microtubule-associated-protein (MAP_2) of high molecular weight (250–350000 daltons) [43] and there is some evidence for analogous phosphorylation of MAP_2 in vivo in fibroblasts [18]. How such a phosphorylation of the microtubule-associated-protein can induce assembly of microtubules is not clear, but it provides a possible mechanism by which cyclic AMP might regulate this assembly, and which might also be operative in the pancreatic B cell.

Calcium at micromolar concentrations is known to inhibit assembly of tubulin into microtubules in vitro [52] and this seems difficult to reconcile with current concepts of an increase in cytosolic calcium concentration as a regulator of the rates of insulin secretion by exocytosis. Again it is not clear whether the effect is on tubulin itself, on another microtubule-associated-protein (MAP_1) or indeed is mediated by another protein which is involved in calcium binding. The calcium-dependent regulatory protein (calmodulin) is obviously a strong candidate for this role. It might be argued that when tubulin is purified from tissues by polymerization/depolymerization cycling, the microtubule-associated-proteins will be selected for and calmodulin selected against. As the degree of purification is increased, then binding of calcium to tubulin and its ability to inhibit polymerization both diminish in parallel so that highly purified tubulin does not itself seem to possess a physiologically important calcium-binding capacity [4].

Microfilaments

In contrast to microtubules, which are known to be affected by a number of drugs, all of which have similar effects on secretion, evidence for the involvement of microfilaments in insulin secretion rests predominantly on the use of a single agent, cytochalasin B, which may induce disruption or hypercontraction of microfilaments in many cell types. It is therefore important to verify that the effects of cytochalasin B are truly specific for microfilaments. The other well established effect of cytochalasin B (in addition to its disruption of microfilaments) is to inhibit glucose transport into some mammalian cells. However, this and other effects of cytochalasin B on B cell metabolism which have been recorded [30], such as inhibition of glucose uptake, glucose oxidation and lactate output, would be expected to inhibit insulin secretion rather than stimulate it as is observed [25]. In addition, cytochalasin D has no effect on glucose transport in the B cell and yet still exerts a stimulatory effect on insulin secretion [31]. It therefore seems reasonable to assume that the observed effects of cytochalasin B are indeed exerted via its action in inhibiting microfilament function. The effects on insulin secretion which have been observed are an increase in glucose [23] and tolbutamide or glucazide-induced secretion [49, 51], together with enhancement of the high rates of secretion induced by glucose plus theophylline. Cytochalasin B also reduces the threshold for glucose stimulation of secretion [49]. Integrity of the microtubule system appears to be required for these effects, since in the presence of vinblastine, cytochalasin B had no stimulatory effect. All of these actions are rapidly reversible on removal of the drug and are dependent on the presence of an adequate concentration of calcium in the incubation medium [51]. Actin is known to be a major constituent protein of microfilaments (Table 1), and the role of actin in B cells has therefore been investigated in some detail.

Actin

An assay for actin is available which has sufficient sensitivity to measure reliably both the globular and filamentous actin content of islet extracts. It utilizes the ability of globular actin to form a complex with the enzyme DNAase I, which results in inhibition of its activity [2]. Thus by estimating the rate of hydrolysis of DNA by DNAase I in the presence of tissue homogenates, it is possible to obtain an estimate of globular actin. After dissociation of the filamentous actin to globular actin by guanidine hydrochloride, it is also possible, using aliquots of the same tissue extract, to obtain estimates of the total actin content of the tissue. This type of assay has been used with islets from the rat [14] and obese mouse [47].

These studies have shown that the total actin content of the tissue represents 0.7%–2% of the total tissue protein, and that 23%–37% of the actin exists in the polymerized form in unstimulated cells. After stimulation of secretion by glucose, however, this proportion is significantly increased to 46%–52% of the total – an approximately one and a half to two fold increase [14]. Enhanced stimulation of secretion by addition of 3-isobutyl-1-methyl xanthine results in a further increase in polymerization to 71%. The glucose stimulation appears to be ATP-dependent since it was inhibited in cells which had been incubated with 2 : 4 dinitrophenol (an uncoupler of oxidative phosphorylation) but was unaltered after omission of extracellular calcium [14]. This last observation precludes the possibility that the increase of actin polymerization is merely a consequence of secretion by exocytosis. These findings suggest that actin exists in B cells in a

dynamic equilibrium between globular and filamentous forms, unlike the situation in muscle where it exists almost entirely as filamentous actin. The ionic conditions in the cell are such that from its known polymerization properties it can be predicted that all of the actin should exist in B cells in the filamentous form. The fact that over 60% of actin in the resting B cell is depolymerized suggests that other interactive proteins are present which bind to globular actin, preventing its polymerization. One such protein is profilin, an actin-binding protein which has been isolated from a number of non-muscle cell types, although not from skeletal muscle. It has a molecular weight of 16000 daltons and it forms a 1 : 1 complex with globular actin [19]. Other potential endogenous actin-binding proteins which have been discussed include spectrin, filamin and DNAase I [19], but neither profilin nor any of the others has been identified in islets of Langerhans, nor indeed has a systematic search for them been carried out.

Myosin

Myosin has been identified as a component of many non-muscle cell types, including rat and catfish islets of Langerhans, by its specific ATPase activity. In islet cells, it appears to be present in concentrations at least 4.5 times those found in rat liver cells [39] (Table 1). Detailed analysis of the protein extracted from catfish islets revealed that myosin constituted 0.97% of cellular proteins and consisted of a heavy chain (200000 daltons) and two light chains (14000 and 19000 daltons), comparable in size to those observed in smooth muscle. However, activation of islet myosin ATPase, unlike that of skeletal muscle, could not be demonstrated on addition of actin, possibly because the myosin light chains as isolated were not phosphorylated [39]. It has been suggested that myosin phosphorylation is an essential prerequisite for actomyosin AT-Pase activation and contraction in smooth muscle and a number of other non-muscle cell types [1]. The role of myosin in the insulin secretory process has been little studied, in part probably because no specific drugs which interfere with its functions are available. However, there is evidence that the actin-myosin complex actomyosin interacts more readily with B granules in vitro than actin alone (see below).

Ultrastructural Aspects

The widespread use of glutaraldehyde as a primary fixative has allowed the visualization of microtubules in the cytoplasm of the B cell where they appear as rod like structures of diameter 22–25 nm. The three dimensional structure of the cell has made it rather difficult to establish comprehensive ultrastructural evidence of the interaction of granules and microtubules, except in monolayer cultures, in which the cells are flattened along one plane. Morphometric analysis of electron micrographs has shown that the proportion of granules which are associated with microtubules did not exceed that expected on the basis of random distribution, even in cells in which secretion was stimulated by glucose [5]. Localization of that pool of tubulin in the cells which is not polymerized into microtubules and which constitutes 60% of the total could be of great interest. Unfortunately, ^{3}H colchicine bound to tubulin does not survive glutaraldehyde fixation so that autoradiographical localization by this means will not be possible, until such time as diffusible compound autoradiography at electron microscope level becomes a practical possibility.

Actin filaments are 5–7 nm in diameter and are distributed in the cytoplasm in some species, as perinuclear bundles of filaments, and as a band of filaments running parallel to and directly beneath the plasma membrane in the so-called 'cell web'. It was proposed by Orci et al. [36] that stimulation of secretion by glucose-induced reorientation and redistribution of this band of filaments in a way which allows improved access of granules to the plasma membrane. However, it seems probable from the frequency with which granules can be observed in close apposition to the plasma membrane, even in unstimulated cells, that the cell web is not a universal barrier preventing access of granules to the membrane. Such a redistribution is also produced by cytochalasin B, resulting in enhanced stimulation of insulin secretion. As an alternative explanation of the re-distribution of the 'cell web' after exposure to cytochalasin B, it has been suggested that the drug causes hypercontraction of the microfilaments, which gives the appearance of aggregates beneath the membrane [33]. The actin content of microfilaments in B cells has been visualized by use of autoantibodies to actin obtained from patients with smooth muscle disease [8]. In principle, it could be demonstrated by decoration with heavy meromyosin if the cells could be rendered permeable to heavy meromyosin without severe disruption of their ultrastructure. The use of high voltage electric discharge may provide a possible way of rendering the cells permeable for this purpose [54]. However, heavy meromyosin decoration will still not provide information about that proportion of actin within the B cells (about 40%) which is in the globular form. Use of antisera to actin labelled with peroxidase [53] might provide a possible approach to the identification of these pools.

Little information is available about the localization of myosin within B cells at the present. This, along with the identification of globular actin and tubulin sub-unit pools, will be essential for the evaluation of the role of contractile-system proteins in insulin secretion.

Cinemicrography

A number of studies have been completed in which the movement of individual particles, presumably granules, have been investigated within islet cells in monolayer culture. By use of time-lapse filming, it was possible to plot the movement of individual particles within the cells. The conclusions of these studies were broadly similar: that the granules move in a discontinuous way by jumps or saltatory movements, and that the speed of movement was in a range 0.8–1.5 μm/s in a pattern which suggests directed background and forward movement along an invisible but oriented pathway [24, 45]. The frequency of such movements was increased by glucose but diminished by adrenaline or the omission of calcium from the incubation media, or by the addition of microtubule inhibitory agents, such as vinblastine or deuterium oxide. This implies that disruption of the microtubules prevents the saltatory movements. However, these two reports differ in one important aspect – in that cytochalasin B is reported either not to affect glucose (8.3 mmol/l) induced saltatory movements of the granules [45], or alternatively to enhance them in the presence of 16.7 mmol/l glucose, as well as to increase the movements seen in the presence of 1.67 mmol/l glucose [24]. The reason for this discrepancy is not clear, but the point is important since an effect of cytochalasin B on saltatory movement implies a direct role of microfilaments as well as of microtubules in the type of granule movement which was observed. Somers et al. [45] also reported effects of cytochalasin B on the cell boundary, presumably by an effect in disrupting or contracting the 'cell web'.

Interaction of Isolated B Granules with Actin, Myosin and Actomyosin

Some indications of the possible interactions between B granules and the contractile proteins within the cells have been obtained by the use of a model system in vitro consisting of isolated B granules and suspensions of these proteins. Interaction or binding of the granules to the proteins is reflected in an alteration of the sedimentation characteristics of the granules. Such an approach has yielded results which indicate that actomyosin is far more effective in promoting binding of granules than actin [13], while myosin is without detectable effect. These interactions were enhanced in the presence of ATP at concentrations expected to be present in the cells, and were unaffected by even high concentrations of calcium [13, 15], possibly because of the intermediary role of calmodulin in mediating effects of calcium in this situation. Ultrastructural studies showed that contacts between granule membranes and actomyosin filaments could be readily observed in this in vitro system [15]. An association of actin and myosin with secretory granule membranes has been observed in adrenal medulla [3], while interactions of isolated pituitary secretory granules with actin but not with myosin, probably by lipophilic mechanisms, have also been reported [38].

Calmodulin has been identified in islet cells [16, 46, 48], and a specific inhibitor of its calcium-binding function (trifluoperazine) has been used to study its role in the insulin secretory process [10, 44, 46]. Effects of trifluoperazine have also been observed in inhibiting activation of cyclic nucleotide phosphodiesterase [46], inhibiting calcium-dependent protein kinase activity [9, 44] and activating calcium-dependent adenylate cyclase activity [48]. Amongst the most interesting of the known aspects of calmodulin action in B cells is its role in the stimulation of protein phosphorylation. The major substrate for calcium-dependent phosphorylation in broken cell preparations of isolated rat islets has been shown to be a 53 000 dalton protein of unknown identity [9]. It will also be important to try to determine the endogenous substrates for this calcium-dependent kinase activity in intact B cells. In view of the reported activation of actomyosin ATPase and of microtubule assemby/disassembly [4] by calmodulin in many cell types, it is tempting to speculate that calmodulin might be associated both with microtubules and with actomyosin in the B cell, thereby providing a link between the increase in cytosolic calcium concentrations and granule movement. If, as is implied from the isolated granule studies, an actin-myosin complex (actomyosin) rather than actin is the active constituent of the microfilaments in the microtubule-microfilamentous system, then a possible mechanism for such a link can be readily envisaged (Fig. 1). Thus calmodulin is a sub-unit of the myosin-phosphorylating enzyme myosin light chain kinase, which has recently been identified in isolated rat islets (Ashcroft, personal communication). Activation of this calcium-dependent and highly specific kinase would lead to phosphorylation of myosin, which in turn would promote its ATP splitting and contractile activity in association with actin at the same site.

Stimulus-Secretion Coupling: A Speculation

It now seems possible, in the light of the information so far available, to make a speculative model which may explain the essential links between the stimulation of secretion and the increased movement of granules and their secretion by exocytosis. Such a model is shown in Figure 2. It is widely supposed that glucose increases intracellular free calcium concentration in the B cell, at least in part by altering trans-plasma membrane calcium fluxes. In addition, those stimuli which elevate cyclic AMP levels are seen as contributing to the cytosolic calcium concentration by inducing intracellular redistribution of calcium from organelle-bound, and in particular mitochondrial, pools. In either case, calcium binds to calmodulin which may, as in other cell types, promote tubulin polymerization to microtubules, perhaps in conjunction with enhanced phosphorylation of tubulin or microtubule-associated proteins by cyclic-AMP-dependent-protein kinases. The binding of calcium to calmodulin may also lead inter alia to activation of myosin kinase, to myosin phosphorylation, and its association with filamentous actin in a way which, as indicated above, can facilitate movement of granules as a consequence of its ATP-dependent contractile activity. Following actomyosin contraction the granules may be expected to move along oriented pathways which are determined by the polymerized microtubules. Transfer of the granules via the microtubule-microfilamentous system may be expected to bring them very close to the plasma membrane, to a distance presumably determined by the balance between electrostatic repulsion of granule and plasma membranes and Van de Waal's attractive forces [5, 11]. The final fusion process may in turn be independently regulated, again possibly as a result of local changes in calcium concentration close to the plasma membrane and of re-orientation of microfilaments in the cell web.

This model cannot of course account even for the information so far available, but hopefully it will provoke some further experiments into aspects of stimulus-secretion coupling of the B cell. It is now 14 years since the 'microtubule-microfilament hypothesis' was proposed; hopefully it may have given way in less

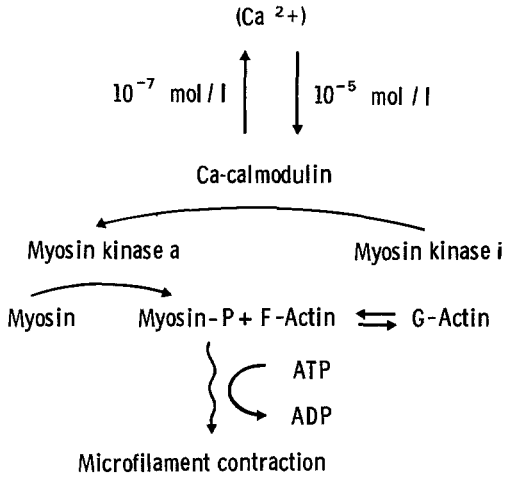

Fig. 1. A possible direct link between cytosolic calcium concentrations and microfilament activation. Myosin kinase a and i represent the active and inactive forms of myosin light chain kinase respectively. Myosin-P indicates the phosphorylated form of myosin. F-actin = filamentous actin. G-actin = globular actin. For details see text

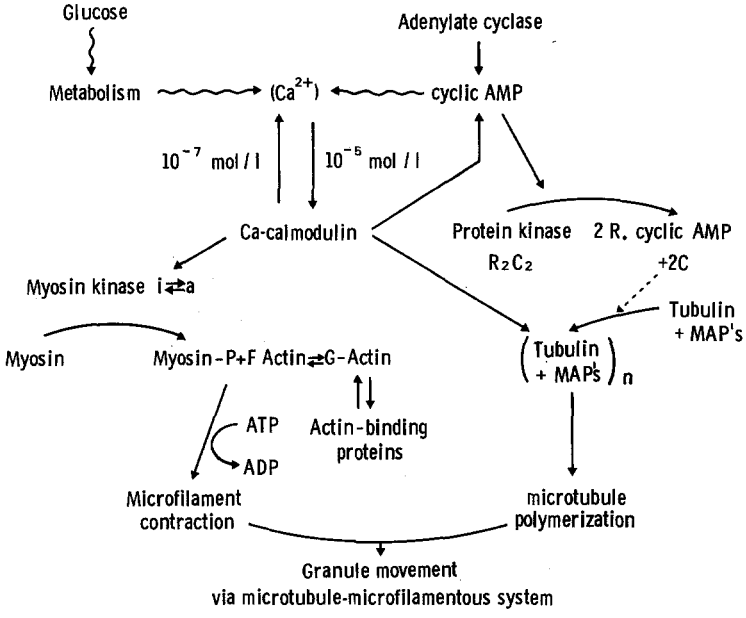

Fig. 2. Some possible relationships between calcium and tubulin, actin and myosin in the initiation of granule movement. R and C represent the receptor and catalytic sub-units of cyclic-AMP-dependent protein kinase; MAP's = microtubule associated proteins. The existence of actin binding proteins in the B cells is speculative. For details see text

than 14 further years to a detailed understanding in molecular terms of the way in which the signal for the initiation of secretion can be translated into the physical process of granule extrusion by exocytosis.

Acknowledgements. We thank Dr. W. Montague for critically reviewing this manuscript. Studies from this laboratory referred to in this article were supported by grants from the Medical Research Council and the British Diabetic Association.

References

1. Adelstein RS, Eisenberg E (1980) Regulation and kinetics of the actin-myosin-ATP interaction. Annu Rev Biochem 49: 921–956
2. Blikstad I, Markey F, Carlsson L, Persson T, Lindberg U (1978) Selective assay of monomeric and filamentous actin in cell extracts, using inhibition of deoxyribonuclease 1. Cell 15: 935–943
3. Burridge K, Phillips JH (1975) Association of actin and myosin with secretory granule membranes. Nature 254: 526–529.
4. Dedman JR, Brinkley BR, Means AR (1979) Regulation of microfilaments and microtubules by calcium and cyclic AMP. In: Greengard P, Robison GA (eds). Advances in cyclic nucleotide research, vol 11. Raven Press, New York, pp 131–174
5. Dean PM (1973) Ultrastructural morphometry of the pancreatic β-cell. Diabetologia 9: 115–119
6. Dean PM (1975) Exocytosis modelling: an electrostatic function for calcium in stimulus-secretion coupling. J Theor Biol 54: 289–308
7. Eipper BA (1974) Rat brain tubulin and protein kinase activity. J Biol Chem 249: 1398–1406
8. Gabbiani G, Malaisse-Lagae F, Blondel B, Orci L (1974) Actin in pancreatic islet cells. Endocrinology 95: 1630–1635
9. Gagliardino JJ, Harrison DE, Christie MR, Gagliardino EE, Ashcroft SJH (1980) Evidence for the participation of calmodulin in stimulus-secretion coupling in the pancreatic β-cell. Biochem J 192: 919–927
10. Henquin JC (1981) Effects of trifluoperazine and pimozide on stimulus-secretion coupling in pancreatic β-cells. Suggestion for a role of calmodulin? Biochem J 196: 771–780
11. Howell SL, Tyhurst M (1977) Distribution of anionic sites on surface of B cell granule and plasma membranes. A study using cationic ferritin. J Cell Sci 27: 299–301
12. Howell SL, Tyhurst M (1978) Role of microtubules in the intracellular transport of growth hormone. Cell Tissue Res 190: 163–171
13. Howell SL, Tyhurst M (1979) Interaction between insulin-storage granules and F-actin in vitro. Biochem J 178: 367–371
14. Howell SL, Tyhurst M (1980) Regulation of actin polymerization in rat islets of Langerhans. Biochem J 192: 381–383
15. Howell SL, Tyhurst M (1981) Actomyosin interaction with isolated β granules. Diabetologia 21: 284 (Abstract)
16. Hutton JC, Penn EJ, Jackson P, Hales CN (1981) Isolation and characterization of calmodulin from an insulin-secreting tumour. Biochem J 193: 875–885
17. Kemper B, Habener JF, Rich A, Potts JP (1975) Microtubules and intracellular conversion of proparathyroid hormone to parathyroid hormone. Endocrinology 96: 903–912
18. Klein I, Willingham M, Pastan I (1978) A high molecular weight phosphoprotein in cultured fibroblasts that associates with polymerized tubulin. Exp Cell Res 114: 229–238
19. Korn ED (1978) Biochemistry of actomyosin-dependent cell motility. Proc Nat Acad Sci 75: 588–599
20. Lacy PE (1961) Electron microscopy of the beta cell of the pancreas. Amer J Med 31: 851–859
21. Lacy PE, Howell SL, Young DA, Fink CJ (1968) New hypothesis of insulin secretion. Nature 219: 1177–1179
22. Lacy PE, Walker MM, Fink CJ (1972) Perifusion of isolated rat islets in vitro. Participation of the microtubular system in the biphasic release of insulin. Diabetes 21: 987–998
23. Lacy PE, Klein NJ, Fink CJ (1973) Effect of cytochalasin B on the biphasic release of insulin in perifused rat islets. Endocrinology 92: 1458–1468
24. Lacy PE, Finke EH, Codilla RC (1975) Cinemicrographic studies on β granule movement in monolayer culture of islet cells. Lab Invest 33: 570–576
25. Levy J, Herchuelz A, Sener A, Malaisse-Lagae F, Malaisse WJ (1976) Cytochalasin B-induced impairment of glucose metabolism in islets of Langerhans. Endocrinology 98: 429–437
26. Malaisse WJ, Malaisse-Lagae F, Walker MO, Lacy PE (1971) The stimulus-secretion coupling of glucose-induced insulin release. V. The participation of a microtubular-microfilamentous system. Diabetes 20: 257–265
27. Malaisse WJ, Malaisse-Lagae F, Van Obberghen E, Somers G, Devis G, Ravazzola M, Orci L (1975) Role of microtubules in the phasic pattern of insulin release. Ann NY Acad Sci 253: 630–652
28. Malaisse-Lagae F, Greider MH, Malaisse WJ, Lacy PE (1971) The stimulus-secretion coupling of glucose-induced insulin release. IV The effect of vincristine and deuterium oxide on the microtubular system of the pancreatic beta cell. J Cell Biol 49: 530–535
29. Malaisse-Lagae F, Amherdt M, Ravazzola M, Sener A, Hutton JC, Orci L, Malaisse WJ (1979) Role of microtubules in the synthesis, conversion and release of (pro)insulin. A biochemical and radioautographic study in rat islets. J Clin Invest 63: 1284–1296
30. McDaniel ML, King S, Anderson S (1974) Effect of cytochalasin B on hexose transport and glucose metabolism in pancreatic islets. Diabetologia 10: 303–308
31. McDaniel M, Roth C, Fink J (1975) Effects of cytochalasins B and D on alloxan inhibition of insulin release. Biochem Biophys Res Comm 66: 1089–1096
32. McDaniel ML, Lacy PE (1980) Involvement of the microtubule-microfilament system in insulin secretion. In: Cumming IA, Funden JW, Mendelssohn FA (eds), Endocrinology 1980. Australian Academy of Science, pp 595–598
33. Miranda AF, Godman GC, Deitch AD, Tanenbaum SW (1974) Action of cytochalasin D on cells of established lines. J Cell Biol 61: 481–500
34. Montague W, Howell SL, Green IC (1975) Insulin release and the microtubular system of the islets of Langerhans. Identification and characterization of tubulin-like protein. Biochem J 148: 237–243
35. Montague W, Howell SL, Green IC (1976) Insulin release and the microtubular system of the islets of Langerhans: Effects of insulin secretagogues on microtubule subunit pool size. Horm Metab Res 8: 166–169
36. Orci L, Gabbay KH, Malaisse WJ (1972) Pancreatic B cell web: its possible role in insulin secretion. Science 175: 1128–1130
37. Orci L, Amherdt M, Malaisse-Lagae F, Rouiller C, Renold AE (1973) Insulin release by emiocytosis: demonstration with freeze etching technique. Science 179: 82–84
38. Ostlund RE, Leung JT, Kipnis DM (1977) Muscle action filaments bind pituitary secretory granules in vitro. J Cell Biol 73: 78–87
39. Ostlund RE, Leung JT, Kipnis DM (1978) Myosins of secretory tissues. J Cell Biol 77: 827–836
40. Pipeleers DG, Pipeleers-Marichal MA, Kipnis DM (1976) Regulation of tubulin synthesis in islets of Langerhans. Proc Natl Acad Sci USA 73: 3188–3191
41. Pipeleers DG, Pipeleers-Marichal M, Kipnis DM (1976) Mi-

crotubule assembly and the intracellular transport of secretory granules in pancreatic islets. Science 191: 88–90
42. Pipeleers DG, Harnie N, Heylen L, Wauters G (1979) Microtubule interaction in islets of Langerhans. Biochemistry and biophysics of the pancreatic B-cell. In: Malaisse WJ, Taljedal IB (eds) European Workshop Brussels. Georg-Thieme Verlag, Stuttgart, pp 163–167
43. Sandoval IV, Cuatrecasas P (1976) Protein kinase associated with tubulin. Affinity chromatography and properties. Biochemistry 15: 3424–3432
44. Schubart UK, Erlichman J, Fleischer N (1980) The role of calmodulin in the regulation of protein phosphorylation and insulin release in hamster insulinoma cells. J Biol Chem 255: 4120–4124
45. Somers G, Blondel B, Orci L, Malaisse WJ (1979) Motile events in pancreatic endocrine cells. Endocrinology 104: 255–264
46. Sugden MC, Christie MR, Ashcroft SJH (1979) Presence and possible role of calcium-dependent regulator (calmodulin) in rat islets of Langerhans. FEBS Letters 105: 95–100
47. Swanston-Flatt SK, Carlsson L, Gylfe E (1980) Actin filament formation in pancreatic β cells during glucose stimulation of insulin secretion. FEBS Letters 117: 299–302
48. Valverde I, Vandermeers A, Anjaneyulu R, Malaisse WJ (1979) Calmodulin activation of adenylate cyclase in pancreatic islets Science 206: 225–227
49. Van Obberghen E, Somers G, Devis G, Vaughan GD, Malaisse-Lagae F, Orci L, Malaisse WJ (1973) Dynamics of insulin release of microtubular-microfilamentous system. 1. Effect of cytochalasin B. J Clin Invest 52: 1041–1051
50. Van Obberghen E, Somers G, Devis G, Ravazzola M, Malaisse-Lagae F, Orci L, Malaisse WJ (1974) Dynamics of insulin release and microtubular-microfilamentous system. VI. Effects of D_2O. Endocrinology 95: 1518–1528
51. Van Obberghen E, Somers G, Devis G, Ravazzola M, Malaisse-Lagae F, Orci L, Malaisse WJ (1975) Dynamics of insulin release and microtubular-microfilamentous system. VII. Do microfilaments provide the motive force for the translocation and extrusion of beta granules? Diabetes 24: 892–901
52. Weisenberg RC (1972) Microtubule formation in vitro in solutions containing low calcium concentrations. Science 177: 1104–1105
53. Willingham MC, Yamada SS, Davies PJA, Rutherford AV, Gallo MG, Pastan I (1981) Intracellular localization of actin in cultured fibroblasts by electron microscopic immunocytochemistry. J Histochem Cytochem 29: 17–37
54. Yaseen A, Pedley KC, Howell SL (1981) Use of high voltage electric discharge to increase permeability of pancreatic B cells. Diabetologia 21: 345 (Abstract)

Dr. S. L. Howell
Department of Physiology
Queen Elizabeth College
University of London
Campden Hill Road
London W8 7AH, UK