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Stephen John Haslam Ashcroft
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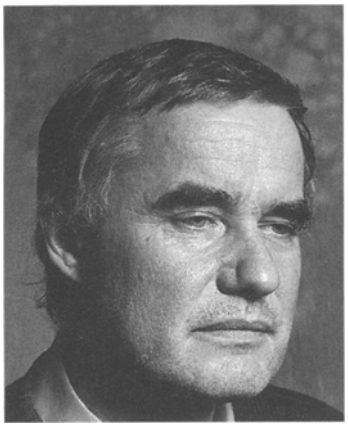

Stephen Ashcroft was born in 1942. He obtained a Ph. D. in 1969 from the University of Bristol and an M. A. in 1976 from the University of Cambridge, UK. Dr. Ashcroft was at the Department of Biochemistry at the University of Bristol from 1965 to 1975 where he was successively: Research Assistant, Research Associate and Lecturer in Biochemistry. From 1975 to 1990 he became a Lecturer in Clinical Biochemistry at the University of Oxford and a Tutor in Biochemistry at Magdalen College Oxford. Since 1990 Dr. Ashcroft has been a Reader in Clinical Biochemistry at the University of Oxford. Dr. Ashcroft's current research activities concern molecular characterisation of the ATP-sensitive K-channel in pancreatic beta cells; the role of protein phosphorylation in the regulation of insulin secretion; regulation of insulin gene expression.

Protein phosphorylation and beta-cell function

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Summary The central role of reversible protein phosphorylation in regulation of beta-cell function is reviewed and the properties of the protein kinases so far defined in beta cells are summarised. The key effect of Ca^{2+} to initiate insulin secretion involves activation of a Ca^{2+}/calmodulin-dependent protein kinase. Potentiation of secretion by agents activating protein kinase A or C appears to involve an increase in the sensitivity of the secretory system to intracellular Ca^{2+}. The effects of MgATP on the binding of [^{3}H]-glibenclamide to the beta-cell sulphonylurea receptor suggest that the properties of this receptor, which controls the activity of ATP-sensitive K-channels, are modulated by phosphorylation. The identity of the kinases and phosphatases responsible is not known but the presence in beta-cell membranes of various kinases not dependent on Ca^{2+} or cyclic AMP, and including tyrosine kinase, is documented, together with the presence of both Ca^{2+}-dependent and Ca^{2+}-independent protein phosphatases. Protein phosphorylation is also involved in regulation of beta-cell Ca^{2+} fluxes and evidence is presented that protein kinase C activation inhibits Ca^{2+} signalling by reducing influx of Ca^{2+} into the beta cell. The identity of the Ca^{2+}/calmodulin-dependent protein kinase activity in beta cells is discussed. Comparison of its properties towards substrates and inhibitors with those of brain Ca^{2+}/calmodulin-dependent protein kinase II suggests that the beta-cell enzyme may be similar or identical to the brain enzyme. Evidence from Northern and Western blotting experiments supports this conclusion. These findings are incorporated in a model for control of insulin secretion. [Diabetologia (1994) 37 [Suppl 2]: S21–S29

Key words Protein kinase, phosphorylation, protein phosphatase, beta cell, insulin secretion, sulphonylurea receptor.

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Abbreviations: K-ATP channels; ATP-sensitive potassium channels; DAG, diacylglycerol; TPA, 12-O-tetradecanoyl phorbol acetate; CaM kinase, calcium/calmodulin-dependent protein kinase.

In the beta cell, reversible protein phosphorylation is implicated in the control of insulin secretion and probably also in insulin biosynthesis. However, the important goal of elucidating which of the 'one thousand and one protein kinases' [1] and 'one thousand and two protein phosphatases' [2] are involved in these functions is far from easy. Approaches taken include i) detection and characterisation of specific protein kinases in the beta cell; ii) determination of the effects of activators and inhibitors of protein kinases on insulin secretion; iii) detection and identification of specific phosphoproteins whose phosphorylation state varies with changes in rates of secretion. In this paper I shall first briefly survey the main conclusions that have been drawn from these studies and then focus on selected areas of our current research.

Beta-cell protein kinases

Protein kinase A

Protein kinase A has been partially purified from rat pancreatic islets and shown to comprise two isoenzymes corresponding to the 'Type I' and 'Type II' holoenzymes found in other tissues [3]. The two isoenzymes differ in their ease of dissociation by histone or NaCl and as in other tissues are likely to share the same catalytic subunit. An M_r of 144,200 was calculated from measurements of sedimentation coefficient and Stokes' radius [3]. Studies with subcellular fractions or intact islets (reviewed in detail in [4]) have demonstrated the existence in beta cells of numerous substrates for protein kinase A, several of which have been identified by more than one laboratory using different procedures. At the present time, however, the nature of the endogenous substrates and their relationship to the secretory process have not been defined.

Although it is well established that glucose elicits a modest increase in beta-cell cyclic AMP [5–8] the mechanism by which this occurs has not been established. Moreover, there is strong evidence that activation of protein kinase A is not necessary for glucose-induced insulin release [9]. The role of cyclic AMP-dependent protein phosphorylation is therefore to mediate the potentiatory effects on glucose-induced insulin release elicited by glucagon, gastric inhibitory peptide or glucagon-like polypeptide-1_{7-36} amide [10].

Protein kinase C

Protein kinase C has been purified to homogeneity from beta cells and shown to be a monomer of M_r 85,200 [11]. The predominant isoform expressed in beta cells of adult islets of Langerhans is protein kinase C-α [12–14]. The presence of other isoforms can be demonstrated by polymerase chain reaction or Northern blotting [15]. Endogenous substrates for protein kinase C can be demonstrated in beta cells [16–18]. Inhibition of protein kinase C by clomiphene has been correlated with changes in phosphorylation of a 37 kDa particulate protein in islets of Langerhans and inhibition of insulin secretion [19]. However, with the exception of MARCKS (myristoylated alanine-rich C-kinase substrate [20]) the identity of the endogenous beta-cell substrates for protein kinase C is unknown and their role in secretion has not been established.

Whether protein kinase C activation is involved in glucose-stimulated insulin release has been controversial (for review see [21]). There is evidence that activation of protein kinase C occurs in islets in response to an increase in glucose concentration [20]. However, it is doubtful whether this increase in activity is of major importance for secretion since most studies find that glucose-induced insulin release is little affected by down-regulation of protein kinase [22–24]. The main role of protein kinase C in insulin secretion may therefore be to mediate the potentiatory effects of agents such as acetylcholine and cholecystokinin which activate phospholipase C and hence increase the beta-cell level of diacylglycerol and IP_3 [10].

Calcium/calmodulin-dependent protein kinases

The beta cell contains a high concentration of calmodulin [25] and inhibitors of calmodulin block insulin secretion [26]. The existence of several beta-cell calcium/calmodulin-dependent protein kinases has been demonstrated. Myosin light chain kinase is present in beta cells [27] and could potentially play a role in granule movement – however, there is no direct evidence for such a mechanism. Calcium/calmodulin-dependent phosphorylation of a peptide of M_r approximately 53 kDa has been widely reported in beta cells [28–30]. Although various suggestions have been made as to the identity of the 53 kDa species, including pyruvate kinase [31] and tubulin [32], it seems most likely to represent autophosphorylation of a kinase (P53 kinase). Since inhibition of P53 kinase by alloxan [33] or dehydrouramil [34] was found to be associated with inhibition of insulin release, a central role for P53 kinase in initiation of insulin secretion has been proposed [29]. The identity of P53 kinase is considered below.

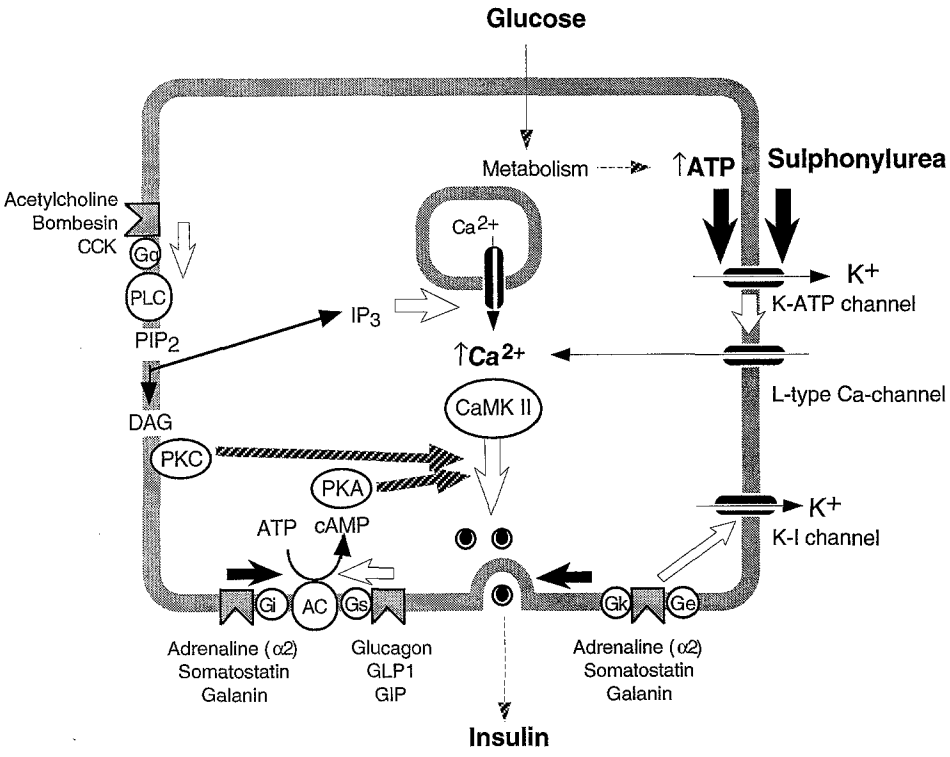

Fig. 1. Protein phosphorylation and insulin secretion. Initiation of insulin secretion by glucose or sulphonylureas involves closure of K-ATP channels and an influx of Ca^{2+} via L-type Ca^{2+} channels. Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II) is suggested to play a key role in mediating the initiation of insulin secretion by Ca^{2+}. Hormones and neurotransmitters have multiple sites of action on the beta cell. Potentiation of secretion (stippled arrows) occurs when protein kinases A or C are activated by cyclic AMP or DAG, respectively. These second messengers are formed in response to activation of adenyl cyclase (AC) or phospholipase C (PLC) by occupation of receptors linked to G_s or G_q. Activation of PLC also liberates IP_3 which releases Ca^{2+} from intracellular stores. Adenycl cyclase can be inhibited by occupation of receptors coupled to G_i. However, inhibitory effects on secretion are also exerted via opening of K-channels and effects on the secretory process itself, both probably involving further G-proteins. Inhibitory effects are indicated by the filled arrows and stimulatory effects by the open arrows

Protein phosphorylation and the beta-cell sulphonylurea receptor

The potency of sulphonylureas in stimulating insulin release parallels their ability to close K-ATP channels in the beta-cell plasma membrane (for review see [35]). Closure of K-ATP channels leads to membrane depolarisation and the resulting influx of Ca^{2+} ions through voltage-dependent Ca^{2+} channels triggers insulin secretion (reviewed in [36]). Glucose, the major physiological regulator of insulin secretion, closes K-ATP channels because increased plasma glucose concentrations cause increased rates of metabolism of the sugar within the beta cell; the resultant increase in intracellular [ATP]/[ADP] blocks K-ATP channel activity. Sulphonylureas, however, close K-ATP channels by directly binding to high affinity sites within the beta-cell membrane. It is currently unclear whether the sulphonylurea-binding sites are identical to the K-ATP channels or whether they are a distinct protein which modulates channel activity.

An inhibitory effect of MgATP has been observed on sulphonylurea binding to beta-cell membranes [37–39]. The nucleotide specificity and requirement for Mg^{2+} of this inhibitory effect of ATP suggested that protein phosphorylation could modulate the properties of the sulphonylurea receptor [38, 39]. This interpretation is supported by the finding that the effect of MgATP on the K_d for binding of [^{3}H]-glibenclamide to mouse islet membranes is not in accordance with a competitive interaction of glibenclamide and MgATP [38].

Since diazoxide inhibits insulin secretion by opening K-ATP channels in pancreatic beta cells [40], it is likely that this sulphonamide also interacts with the sulphonylurea receptor. However, we found that diazoxide, alone, failed to influence [^{3}H]-glibenclamide binding to membranes from HIT T15 beta cells [37]. On the other hand, diazoxide did inhibit [^{3}H]-glibenclamide binding to whole HIT cells and the magnitude of this effect was decreased when the cells were depleted of ATP [37]. Direct evidence that ATP could influence the binding of diazoxide to the sulphonylurea receptor was obtained by showing that diazoxide was able to displace [^{3}H]-glibenclamide from HIT beta-cell [37] or islet [39] membranes in the presence of MgATP. This effect of MgATP was not reproduced by non-metabolizable ATP analogues or by ATP in the absence of Mg^{2+}. In the presence of 1 mmol/l-MgATP, diazoxide inhibited [^{3}H]-glibenclamide binding to HIT cell membranes over the same range of diazoxide concentrations that increased ^{86}Rb efflux from HIT cells [37, 41]. These results suggest that protein phosphorylation can modulate the binding to the sulphonylurea receptor not only of glibenclamide but also of diazoxide.

There are several possible explanations for the modulation by phosphorylation of the effect of diazoxide on [^{3}H]-glibenclamide binding. Firstly, diazoxide may activate a kinase which phosphorylates the sulphonylurea receptor/K-ATP channel. We consider that this is unlikely, since we find that diazoxide retains the ability to inhibit binding to membranes ex-

posed to MgATP even when added after the added ATP has been hydrolysed by endogenous ATPase activity. Furthermore, we find no effect of diazoxide on incorporation of ^{32}P from [γ-^{32}P]-ATP into HIT-cell membrane proteins. Secondly, diazoxide may inhibit a protein phosphatase which dephosphorylates the glibenclamide receptor/K-ATP channel. This also seems unlikely, since diazoxide inhibits [^3H]-glibenclamide binding even in the presence of NaF, an inhibitor of protein phosphatases. Moreover, we find no effect of diazoxide on dephosphorylation of HIT-cell membrane proteins labelled by incubation with [γ-^{32}P]-ATP. We therefore conclude that diazoxide may directly inhibit [^3H]-glibenclamide binding to the receptor by itself binding to the phosphorylated receptor or a related protein(s).

Three findings suggest that the phosphorylation of the receptor necessary for diazoxide to inhibit [^3H]-glibenclamide binding may occur at two different sites. Firstly, the time course for inhibition of [^3H]-glibenclamide binding by MgATP differs from that for inhibition by diazoxide in the presence of MgATP. Secondly, ATP-γS has only a small inhibitory effect on binding but permits sustained inhibition by diazoxide. Thirdly, although inhibition of [^3H]-glibenclamide binding by MgATP can also be observed for the solubilised receptor, we do not see the revealing effect of MgATP on diazoxide-evoked inhibition of glibenclamide binding to the solubilised receptor, suggesting that the inhibitory and revealing effects of MgATP are mediated by distinct pathways.

These findings require that beta-cell membranes contain both protein kinase(s) and protein phosphatase(s) active under the conditions of our binding assays. Since the latter are conducted in the absence of added Ca^{2+} or cyclic AMP the protein kinases are presumably distinct from those discussed above. We have begun to characterise these activities. When HIT T15 beta-cell membranes were incubated with [γ-^{32}P]-ATP there was substantial incorporation of ^{32}P into membrane proteins. Phosphorylation was maximal after 5 min and declined thereafter. Since exogenous kinases were not added to the incubation buffer, these data indicate the presence of endogenous kinases in HIT beta-cell membranes. Furthermore, since the incubation medium contained 0.25 mmol/l-EGTA, it appears that the kinase(s) involved are not strongly Ca^{2+}-dependent.

The decline in phosphorylation is indicative of endogenous phosphatase activity; after 5 min, the [γ-^{32}P]-ATP in the incubation mixture was completely hydrolysed by endogenous ATPase activity and rephosphorylation could therefore not occur. Since dephosphorylation occurs in the absence of Ca^{2+}, the phosphatases involved also do not have a high Ca^{2+}-requirement.

In the absence of Mg^{2+}, most of the incorporation of radioactivity was not evident, confirming that it results from protein phosphorylation. In the absence of Mg^{2+}, a protein of molecular weight 45 kDa was strongly labelled and there was weak labelling of a 120 kDa band; in the additional presence of 2 mmol/l-EDTA, the latter band was shifted to 115 kDa. We used [α-^{32}P]-ATP to investigate whether these proteins were labelled with ^{32}P by phosphorylation or by some other mechanism which involved covalent incorporation of ^{32}P-ATP. After incubation with [α-^{32}P]-ATP in the presence of Mg^{2+}, radioactivity was found in an 80 kDa band. The intensity of this band was reduced in Mg^{2+}-free solution and was absent in the additional presence of 2 mmol/l-EDTA, suggesting that the 80 kDa protein is covalently labelled by MgATP but not by free ATP. In the presence of EDTA and EGTA, label was incorporated into an additional 135 kDa band, suggesting that this protein is covalently labelled by free ATP. This 135 kDa protein may correspond to the 120/115 kDa band found with [γ-^{32}P]-ATP in Mg^{2+}-free solution consistent with labelling of this protein by ^{32}P-ATP and not by ^{32}P-phosphate. No label was found in a 45 kDa protein with [α-^{32}P]-ATP. This result suggests that the 45 kDa protein, labelled after incubation in Mg^{2+}-free solution with [γ-^{32}P]-ATP, is labelled with ^{32}P-phosphate and not with [^{32}P]-ATP. This result may indicate Mg^{2+}-independent phosphorylation of the 45 kDa protein.

We also examined the effects of protein kinase inhibitors on the incorporation of [γ-^{32}P] into HIT beta-cell membranes. Protein kinase A inhibitor peptide (10 μmol/l) slightly reduced labelling of a 100 kDa band but was without effect on most labelled species. Protein kinase C inhibitor peptide (10 μmol/l) was without effect. Tyrphostin (100 μmol/l), an inhibitor of tyrosine kinases, almost completely abolished phosphorylation of an 80 kDa band and decreased labelling of bands at 115 and 68 kDa. These results suggest that protein kinase A and tyrosine kinase activity are present in HIT-cell membranes but that substrate proteins are few.

The effects on dephosphorylation of HIT beta-cell membranes of a number of agents known to inhibit or activate protein phosphatases were also studied. In these experiments, membranes were first phosphorylated for 5 min. A sample was removed and the membranes incubated for a further 30 min in the presence or absence of various inhibitors or activators. The non-specific phosphatase inhibitors NaF and Na_3VO_4 had marked effects on dephosphorylation of HIT beta-cell membrane proteins. NaF caused marked preservation of label in several proteins, particularly those of 45, 50, 54 and 57 kDa. Na_3VO_4 also strongly decreased dephosphorylation of the proteins affected by NaF. In addition, it is of interest that phosphorylation of a 115 kDa species, whose phosphorylation was reduced by tyrphostin, was markedly and specifically enhanced by 1 mmol/l-

Na_3VO_4, which, at this concentration, inhibits phosphotyrosine phosphatases. This observation provides further evidence that the 115 kDa substrate protein is phosphorylated on tyrosine residues.

Microcystin produced a dose-dependent inhibition of dephosphorylation of 45, 50, 54, 60, 68 and 135 kDa bands, suggesting the presence of membrane-bound protein phosphatases 1 and/or 2A; the 115 kDa protein was unaffected. Addition of 5 mmol/l-$CaCl_2$ markedly potentiated dephosphorylation of a 45 kDa protein suggesting the additional presence of an endogenous Ca^{2+}-dependent phosphatase; dephosphorylation of most bands was not affected by calcium.

These observations indicate that beta-cell membrane fractions contain numerous protein kinase and phosphatase activities potentially capable of modifying K-ATP channel activity and other beta-cell membrane functions. There is evidence from secretion studies that tyrosine kinase activities may have functional importance for the beta cell [42, 43].

Protein kinase C, Ca^{2+} and insulin secretion

Acetylcholine causes a sustained potentiation of glucose-stimulated insulin release [24]. The effect is mediated by M_2-muscarinic receptors [44] coupled to phospholipase C which generates an increase in DAG and IP_3. The former will activate protein kinase C while the latter can evoke mobilisation of Ca^{2+} from intracellular stores. However, we find that the transient increase in intracellular Ca^{2+} induced by acetylcholine is prevented in the absence of extracellular Ca^{2+} or when influx of Ca^{2+} is blocked by verapamil [24]. Hence Ca^{2+} entry, rather than Ca^{2+} mobilisation, may be the main contributor to the acetylcholine-evoked increase in intracellular Ca^{2+}. This increase is, however, relatively modest and transient. Therefore, the activation of protein kinase C by DAG may be of more importance for the increase in insulin release. We have examined this possibility further by examining the effects of inhibition or down-regulation of protein kinase C on potentiation of insulin release by acetylcholine [24]. HIT T15 beta cells were exposed to a phorbol ester, TPA, for 24 h which resulted in a 73 % decrease in the activity of protein kinase C. Down-regulation of protein kinase C had no significant effect on either the sustained rise induced by glucose or the transient increase in intracellular [Ca^{2+}] induced by acetylcholine. However, insulin release was markedly affected by pretreatment with TPA; the secretory response to glucose was somewhat reduced and the potentiation by acetylcholine completely abolished. In contrast the potentiation induced by activating protein kinase A by the addition of forskolin was not reduced by preexposure to TPA. These findings suggest that acetylcholine potentiates insulin secretion by a two-step mechanism. An initial increase in intracellular Ca^{2+} as a consequence of enhanced influx through voltage-dependent Ca^{2+} channels stimulates insulin release and primes the secretory mechanism for protein kinase C activation; this in turn sensitises the secretory mechanism to Ca^{2+}, causing sustained insulin release.

The above studies provided no evidence for a stimulatory effect of protein kinase C on intracellular Ca^{2+}. Further studies have shown that protein kinase C activation is actually associated with a *lowering* of intracellular Ca^{2+}. In a first series of experiments we subjected HIT T15 beta cells to step-wise increases in extracellular Ca^{2+} [45]. In the presence of a stimulatory concentration of glucose, this resulted in stepwise increases in intracellular Ca^{2+}. TPA had no effect on intracellular Ca^{2+} in the absence of glucose but, in the presence of 10 mmol/l glucose, TPA lowered the intracellular Ca^{2+} by around 20 % at all extracellular Ca^{2+} concentrations greater than 0.25 mmol/l. Despite lowering intracellular Ca^{2+}, TPA potentiated insulin secretion at all extracellular Ca^{2+} concentrations tested. It was also noted that TPA lowered the threshold extracellular Ca^{2+} concentration at which glucose stimulated insulin release.

In a second series of experiments [46] we studied the effects of vasopressin. Vasopressin was shown to elicit marked insulin release from HIT T15 beta cells. As for acetylcholine this stimulation was dependent on the presence in the extracellular medium of both glucose and Ca^{2+}. Vasopressin also evoked a marked, concentration-dependent increase in intracellular Ca^{2+} which was biphasic – an initial spike was followed by a sustained elevation. This increase also required glucose and was blocked in the absence of extracellular Ca^{2+} or presence of verapamil. Down-regulation of protein kinase C had no effect on the vasopressin-induced increases in insulin or Ca^{2+} indicating that protein kinase C has no direct role in vasopressin-induced insulin release. However, short-term exposure to TPA markedly reduced the steady-state level of Ca^{2+} attained in the presence of 2 mmol/l glucose and totally abolished the rise in intracellular Ca^{2+} elicited by vasopressin. Evidence that this effect of TPA was indeed mediated by protein kinase C was provided by the observations that the inhibitory action could be prevented both by down-regulation of protein kinase C or by inhibition of protein kinase C with staurosporine. Short-term exposure to TPA did not, however, inhibit vasopressin-induced insulin release. These findings suggest that protein kinase C activation inhibits Ca^{2+} signalling by reducing influx of Ca^{2+} into the beta cell. Despite this effect on intracellular Ca^{2+}, which has also been observed in mouse islets [47], insulin secretion is enhanced by activation of protein

kinase C. We interpret this as indicating that protein kinase C activation stimulates insulin release primarily by sensitising the beta-cell secretory system to Ca^{2+}.

Calcium/calmodulin-dependent protein kinase II in the beta cell

In several respects, the beta-cell CaM kinase that phosphorylates an endogenous beta-cell protein of M_r 53 kDa resembles CaM kinase II, a multifunctional CaM kinase. CaM kinase II (for review see [48]) is an oligomeric protein of M_r around 500–600 kDa, composed of distinct but related subunits of 50 to 60 kDa in various ratios. The enzyme is widely distributed and shows a broad substrate specificity. Both the α and β subunits can be autophosphorylated in a Ca^{2+}/calmodulin-dependent manner resulting in the appearance of Ca^{2+}-independent kinase activity. The cDNAs for both α and β subunits have been cloned and the deduced amino acid sequences described [49–50]. These subunits are highly homologous. Further homologous subunits (β', γ and δ) have also been identified by molecular cloning [51]. A major substrate for CaM kinase II in nerve tissue is synapsin I which is bound to a high affinity site on synaptic vesicles [52] and may link the vesicles to the cytoskeleton [53]. Phosphorylation of synapsin I by CaM kinase II reduces the affinity of this interaction [54] and a role for this phosphorylation in neurotransmitter release postulated [55]. Direct evidence for this came from experiments on isolated nerve terminals. When CaM kinase was activated by thiophosphorylation and introduced into rat brain synaptosomes, enhanced rates of release of glutamate and noradrenalin were observed together with increased phosphorylation of synapsin I [56]. Further evidence was obtained by introducing a specific peptide inhibitor of CaM kinase into synaptosomes; the rate of glutamate release was significantly inhibited.

In view of the M_r of the beta-cell CaM kinase, its aggregation under non-denaturing conditions, its association with the cytoskeleton and its autophosphorylation we have investigated the possibility that the beta-cell enzyme may be CaM kinase II.

It has been shown that CaM kinase activity in homogenates of brain and islets is susceptible to inhibition by alloxan [33]. We showed that dehydrouramil, an analogue of alloxan, also inhibits beta-cell CaM kinase at concentrations of dehydrouramil that produced a marked inhibition of insulin release [34]. We then compared the substrate specificity of CaM kinase activity in extracts of islets with purified brain CaM kinase II [57]. Islet CaM kinase phosphorylated major endogenous substrates of 102, 57 and 53 kDa and also exogenous glycogen synthase: brain CaM kinase II phosphorylated glycogen synthase and peptides of 57 and 53 kDa. Alloxan (1 mmol/l) inhibited the phosphorylation of glycogen synthase and the 102, 57 and 53 kDa islet peptides by islet CaM kinase; the phosphorylation of glycogen synthase and the 57 and 53 kDa substrates by brain CaM kinase II was also inhibited by alloxan. The 102 kDa substrate was located in the post-100,000 g supernatant and the 57 and 53 kDa substrates in a particulate fraction. These data suggest that islet CaM kinase is similar to, if not identical with, brain CaM kinase II.

We have now obtained direct evidence that beta cells contain CaM kinase II from Northern and Western blotting [58]. Northern blots of RINm5F beta cells with a riboprobe for brain CaM kinase II α-subunit reveal a single band of 4500 bp. We also find, in agreement with [59], that antibodies to a peptide corresponding to residues 281–309 of brain CaM kinase II detect a band of approximately 50 kDa on Western blots of islets that corresponds to a peak of Ca^{2+}/calmodulin-dependent phosphorylation.

Evidence for involvement of CaM kinase in insulin secretion is provided by the demonstration that loss of Ca^{2+}-induced insulin release from permeabilised islets is accompanied by loss of Ca^{2+}-induced protein phosphorylation [60]. Inhibition of glucose-stimulated insulin release by the CaM kinase II inhibitor KN-62 [61] is consistent with a role for the kinase in glucose-induced insulin release. However, this finding is not conclusive since KN-62 may also have a direct effect on L-type Ca-channels in beta cells [62]. Stronger evidence that CaM kinase II mediates the stimulatory effect of glucose on insulin secretion has come from the demonstration that exposure to increased glucose concentrations increased the extent of autophosphorylation of islet CaM kinase II detected by immunoprecipitation [63]. That inhibition of beta-cell CaM kinase results in inhibition of insulin secretion has also been shown at the single cell level by the patch clamp technique [64]. Exocytosis from single beta cells was followed by measuring the capacitance of the plasma membrane. Capacitance is proportional to surface area which increases when secretory vesicles fuse with the plasma membrane: changes in cell capacitance may thus be used to monitor exocytosis. It was shown that a specific peptide inhibitor of CaM kinase II (residues 290–309) markedly reduced secretion when included in the pipette solution without affecting the Ca^{2+} current. Therefore, beta-cell CaM kinase II regulates insulin secretion distal to the elevation of intracellular Ca^{2+}.

The different Ca^{2+} and calmodulin-dependencies of phosphorylation of the endogenous islet substrates suggested that a distinct kinase was involved in phosphorylating the 102 kDa species [57]. This kinase probably corresponds to CaM kinase III and the 102 kDa substrate to elongation factor 2 since the 102 kDa peptide was shown to undergo ADP-

ribosylation in the presence of diphtheria toxin and NAD^+ [57]. A role for CaM kinase III in regulation of insulin biosynthesis thus seems possible.

Conclusions

Figure 1 depicts a model for control of insulin secretion incorporating some of the findings discussed here. Protein phosphorylation is suggested to play a central role in regulation of beta-cell function with CaM kinase II mediating a key triggering effect of Ca^{2+} on secretion. Potentiation of secretion by agents activating protein kinase A or C appears to involve an increase in the sensitivity of the secretory system to intracellular Ca^{2+} [44, 45, 65, 66].

The situation is undoubtedly more complex than shown in this model. Thus, protein kinase A and protein kinase C activation can also modulate beta-cell ion fluxes both positively and negatively [47, 67, 68]. The beta-cell also contain, numerous so far uncharacterised protein kinases. These can be detected in membrane fractions and include tyrosine kinase activity. Interestingly, the tyrosine kinase inhibitor genistein has been shown to block interleukin 1β-induced inhibition of insulin secretion and formation of nitric oxide in beta-cells [42]. Tyrosine kinase inhibitors also inhibit glucose-induced insulin release [43]. Arachidonic acid and some other fatty acids activate a beta-cell protein kinase distinct from protein kinase A or C [69]. Extracts of beta cells can also be shown to contain protein phosphatase activities, and in permeabilised islets it has been shown that the protein phosphatase inhibitor okadaic acid enhanced both basal and cyclic AMP-induced insulin secretion and protein phosphorylation [70]. The relevant protein phosphatases have not yet been characterised.

Perhaps the greatest limitation of current knowledge is that the phosphoprotein substrate(s) relevant to secretion have not been defined and exocytosis remains a poorly understood process. Future progress requires molecular characterisation of the components of the secretory machinery.

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