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Review Articles

Glucoreceptor Mechanisms and the Control of Insulin Release and Biosynthesis*

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Summary. The models proposed for the means whereby the B-cell recognises glucose and related compounds as signals for insulin release and biosynthesis are discussed. The observed correlations between rates of metabolism and insulin release and biosynthesis are consistent with the substrate-site hypothesis. For glucose itself, the enzymes catalysing the phosphorylation of the sugar provide an explanation for the major characteristics of the islet responses, but for N-acetylglucosamine evidence is presented that the sugar transport system fulfils this discriminatory role. Possible mechanisms whereby sugar metabolism may be linked to changes in Ca^{2+}-handling are considered and evidence is given supporting a role for the cytosolic NADPH/$NADP^+$ ratio and the islet content of phosphoenolpyruvate. The nature of the targets for cyclic AMP and Ca^{2+} is discussed and some properties of islet cAMP-dependent protein kinase are summarised. Evidence is presented for the presence of calmodulin in islets and the possible involvement of calmodulin in stimulus-secretion coupling. On the basis of these considerations a speculative hypothesis for the mechanisms involved in the B-cell responses to glucose is outlined.

The mechanisms whereby glucose stimulates the biosynthesis of insulin and its secretion from the pancreatic B-cell have been extensively studied since the introduction of methods for the isolation of viable islets of Langerhans [1, 2, 3]. The conceptual foundations for the approaches used in these investigations were however laid by earlier in vitro studies on insulin release from pancreas preparations [4, 5]; examination of the specificity of the insulin secretory response to sugars suggested a relationship between sugar metabolism and insulin release and led to the formulation of a fruitful hypothesis [6] subsequently to be explored fully with isolated islets as described in this paper. A requirement for extracellular Ca^{2+} for stimulation of insulin release [7, 8] suggested a key role for Ca^{2+} in stimulus-secretion coupling in the B-cell as in other tissues: this concept has been fully confirmed [9–12]. The potentiatory effects of glucagon on insulin release [13] also indicated that cAMP may modulate the secretory process.

In this paper I shall discuss studies with isolated islets of Langerhans which have sought to explore the actions of glucose on the B-cell by considering the following questions:

I) How is glucose recognised as a signal for insulin biosynthesis and release?

II) How does recognition of glucose lead to changes in intracellular Ca^{2+}?

III) How may Ca^{2+}, cAMP and other factors interact with regulatory components of the secretory and biosynthetic machinery?

These studies have been conducted with islets isolated by a collagenase method from rat or mouse pancreas. The islets of these species contain predominantly B-cells, and it is an assumption and limitation of the studies that the metabolic behaviour of the islets reflects essentially that of the B-cells: it has not thus far proved practicable to study pure preparations of B-cells. The methods used in these studies are to be found in the references cited.

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Table 1. Metabolism of sugars by islets and stimulation of insulin release and biosynthesis

Sugar	Response		
	Metabolism	Insulin release	Insulin biosynthesis
Glucose	+	+	+
N-acetylglucosamine	+	+	+
N-acetylmannosamine	−	−	−
Allose	−	−	−
Altrose	−	−	−
Arabinose	−	−	−
2-Deoxyglucose	−	−	−
Fructose	±	±	±
Fucose	−	−	−
Galactose	−	−	−
L-Glucose	−	−	−
Gulose	−	−	−
Idose	−	−	−
Mannose	+	+	+
3-Methylglucose	−	−	−
Ribitol	−	−	−
Ribose	−	−	−
Sorbitol	−	−	−
Sucrose	−	−	−
Talose	−	−	−
Xylitol	−	−	−

The table compares the ability of sugars to serve as metabolic substrates for islets and to stimulate insulin release and biosynthesis. The data are taken from [14–17]. All sugars are the D-stereoisomers unless stated otherwise

How Is Glucose Recognised as a Signal by the B-Cell?

Glucoreceptor Models

Two models have been formulated for the glucoreceptor mechanism [6]. In the regulator-site model, the recognition system is envisaged as a protein located presumably on the cell membrane and capable of binding glucose. A resulting conformational change in this protein initiates the events leading to insulin release and biosynthesis. On the alternative 'substrate-site' model, it is the metabolism of glucose within the B-cell that generates a change in concentration of one or more key metabolic intermediates that acts as an intracellular trigger for insulin release and biosynthesis. The approach to distinguishing these models has, broadly, been to devise methods for the measurement of various metabolic parameters in islets and to determine whether parallel rates of sugar metabolism and of insulin release and biosynthesis are found. The substrate-site hypothesis would be invalidated and the regulator-site model favoured by any instance of non-parallel behaviour e.g. significant metabolism of a sugar that failed to stimulate insulin release/biosynthesis; stimulation of insulin release/biosynthesis by a non-metabolized sugar; inhibition of sugar metabolism without impairment of secretion or biosynthesis.

Islet Metabolism and the Release and Biosynthesis of Insulin

Table 1 summarises the results of detailed studies [14–17] on the ability of glucose analogues to stimulate insulin release and biosynthesis and to be metabolized by pancreatic islets. Metabolism has been assessed in various ways including $^{14}CO_2$ evolution from ^{14}C-labelled sugar as an index of oxidation; ^3H_2O production from ^3H-labelled sugar as an estimate of glycolysis; ability to maintain ATP levels as a measure of fuel function; lactate output. Of the wide range of sugars and derivatives tested, only glucose itself, mannose and N-acetylglucosamine are well-metabolized by islets and these are the only sugars we have found to be capable of eliciting insulin release and biosynthesis. The range of glucose analogues ineffective as stimulants and as substrates for metabolism is impressive and includes, for instance all six D-aldohexoses apart from glucose and mannose [16]. Fructose deserves comment: ineffective alone as a stimulant, fructose nevertheless stimulates both insulin release [15] and biosynthesis [16] in the presence of a non-stimulatory concentration of glucose. This apparently anomalous behaviour has been rationalized by studies on fructose metabolism [18] in which it was shown that rates of fructose metabolism never exceed those seen with sub-stimulatory glucose concentrations: however the combined fluxes of fructose and low glucose fall within the range of values at stimulatory glucose concentrations. These data then support the substrate-site hypothesis.

A further aspect of specificity arises from consideration of potential metabolites that may enter carbohydrate metabolism at a level other than hexose-P. The compounds studied are glyceraldehyde and dihydroxyacetone which may enter glycolysis at the triose-P level and inosine which can also give rise to triose-P after first being converted to hypoxanthine and ribose 1-P. It is found that all these agents are substrates for B-cell metabolism and all elicit insulin release and biosynthesis [14, 16, 19, 20]. These data, summarized in Figure 1, are in accord with the view that the substrate-site hypothesis may apply both to insulin release and insulin biosynthesis.

The question of whether metabolic rates vary over those concentrations of sugar that stimulate release and biosynthesis of insulin has been most

extensively investigated for glucose and N-acetylglucosamine [15, 16, 21, 22, 23]. There is in general excellent agreement for both these sugars between rates of metabolism and rates of insulin release and biosynthesis as a function of sugar concentration. One possible exception is that the threshold for stimulation of insulin biosynthesis by glucose is marginally lower than for glucose utilization or glucose-stimulated insulin release [15].

The actions of a number of metabolic inhibitors have been studied. The findings further support the substrate-site hypothesis. Thus mannoheptulose inhibits only the metabolism of glucose and mannose;

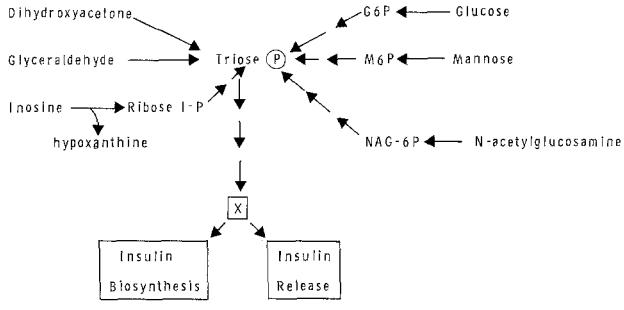


Fig. 1. Metabolites stimulating insulin release and biosynthesis. For details see [14, 16, 19, 20]

and only insulin release or biosynthesis induced by these sugars [1, 16]. Phloretin, on the other hand, inhibits preferentially N-acetylglucosamine metabolism and effects of N-acetylglucosamine on release and biosynthesis of insulin [16, 23, 24, 25]. It is also now generally agreed [26], in contrast to an earlier claim [27], that inhibition of sugar metabolism by iodoacetate is accompanied by parallel inhibition of insulin release.

These data, together with findings such as the anomeric specificity of glucose metabolism [28] and the stimulatory effect of glycogenolysis on insulin release in glycogen-loaded islets [29], constitute the major part of the case for the substrate-site hypothesis. They do not exclude the possibility that direct actions of glucose itself may also be involved. Studies in support of this possibility are described elsewhere [30, 31, 32]. Nevertheless they provide strong support for a major role for glucose metabolism in glucose-stimulated release and biosynthesis of insulin.

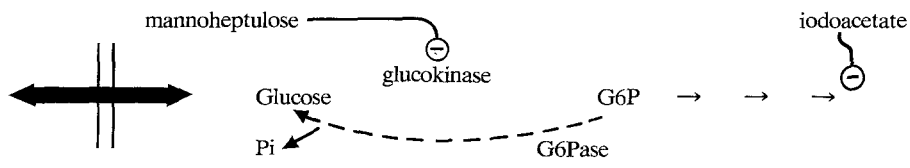
Therefore the 'glucoreceptor' is identified with the rate-limiting step for glucose utilization. Available evidence suggests strongly that for the pancreatic islets the rate-limiting step for glucose utilization is phosphorylation of the sugar. Data for parameters of glucose metabolism are summarised in Table 2(a).

Table 2. Metabolic recognition of glucose

(a)

Process	Kinetics		Effects of inhibitors		
	Km (mmol/l)	Vmax	phloretin	manno-heptulose	iodoacetate
Transport	50	1600 pmol/h/islet	↓	○	○
Phosphorylation	16	51 pmol/h/islet	○	↓	○
Utilization	7	42 pmol/h/islet	○	↓	↓
Oxidation	7	22 pmol/h/islet	○	↓	↓
Insulin release	10	∿ 10-fold	○	↓	↓
Insulin biosynthesis	7	∿ 10-fold	○	↓	↓

(b)



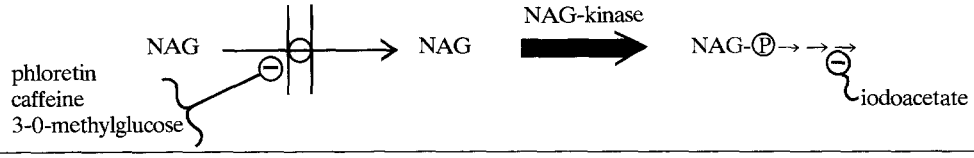
In (a) are shown values for rates of initial steps of glucose metabolism in islets and the effects of inhibitors (↓ = inhibition; ○ = no inhibition). The data are taken from [16, 26, 30–32, 34]. In (b) are shown the conclusions derived from these data. ◗ represents a rapid non-rate-limiting step; → represents a rate-limiting step. ⊖ indicates a site of inhibition

Table 3. Metabolic recognition of N-acetylglucosamine (NAG)

(a)

Process	Kinetics		Effects of inhibitors				
	Km (mmol/l)	Vmax	phloretin	mannoheptulose	iodoacetate	caffeine	3-0-MG
Transport	–	–	(↓)	(○)	(○)	(↓)	(↓)
Phosphorylation	0.02	200 pmol/h/islet	○	○	(○)	(○)	(○)
Utilization	–	34 pmol/h/islet	(↓)	○	↓	–	–
Oxidation	26	21 pmol/h/islet	↓	○	↓	↓	↓
Insulin release	17	∿ 4-fold	↓	○	↓	↓	↓
Insulin biosynthesis	15	∿ 5-fold	↓	○	–	↓	–

(b)



In (a) are given values for rates of initial steps of NAG metabolism by islets and the effects of inhibitors (↓ = inhibition; ○ = no inhibition; – = not measured. Symbols in parenthesis represent inferences rather than direct measurement). The data are taken from [16, 23, 24, 42]. In (b) are shown the conslusions derived from these data: same symbols as in Table 2(b). NAG, N-acetylglucosamine; 3-0-MG, 3-0-methylglucose

The rate of transport of glucose is very much faster than the rate of glucose utilization and the maximal amount of glucose-phosphorylating activity [31, 33, 34]. The latter is just sufficient to account for the maximum rate of glucose utilization. Intracellular glucose concentrations are the same as extracellular [35]. Glucose utilization and oxidation is inhibited by mannoheptulose but not by phloretin although the latter markedly inhibits transport of glucose into islets [36]. Moreover the Km for transport of glucose is well above that for utilization of the sugar. These data identify the phosphorylation of glucose as the rate-limiting step for its metabolism [Table 2(b)].

Studies on mouse and rat islet extracts [34, 35, 37] have identified D-glucose-ATP-phosphotransferase activity with a Km for glucose of 16 mmol/l. This enzyme appears to be distinct from hexokinase (Km for glucose 0.1 mmol/l) since it is not inhibited by glucose 6-phosphate and may be similar to liver glucokinase: this conclusion remains tentative pending separation and purification. In addition, glucose 6-phosphatase inhibited by glucose has been demonstrated in extracts of mouse islets [34, 38]. Glucose phosphorylation is inhibited competitively by mannoheptulose [37]. Thus, the properties of the enzymes involved in the phosphorylation of glucose by islets (reviewed in [39, 40]) provide an explanation for the concentration dependence and inhibition by mannoheptulose of islet glucose utilization and, on the substrate-site hypothesis, may constitute the glucose-sensor mechanism.

However it is of conceptual importance to note that such enzymic activity fails to explain islet responses to N-acetylglucosamine. Most obviously N-acetylglucosamine metabolism and N-acetylglucosamine-stimulated release and biosynthesis of insulin are not inhibited by mannoheptulose [16, 26, 41, 42]. Data which resolve this problem are summarized in Table 3(a). It was found that phosphorylation of N-acetylglucosamine by islets was catalysed by a specific N-acetylglucosamine kinase which was not inhibited by mannoheptulose [43]. However this enzyme had a Km for N-acetylglucosamine of 0.02 mmol/l, several orders of magnitude lower than the Km for N-acetylglucosamine-stimulated release and biosynthesis. Metabolism of N-acetylglucosamine had a high Km for N-acetylglucosamine, and the Vmax of N-acetylglucosamine utilization was much lower than the maximal extractable activity of N-acetylglucosamine kinase [24, 43]. These findings suggested that, in contrast to glucose, the rate-limiting step for N-acetylglucosamine metabolism (and hence the 'N-acetylglucosamine receptor') was transport into the cell.

This possibility was supported by the observed effects of three agents. Firstly, phloretin was found to

be a potent inhibitor of islet N-acetylglucosamine utilization and of N-acetylglucosamine-stimulated insulin release and biosynthesis [16, 23, 24, 30]. Secondly, caffeine inhibits the oxidation of N-acetylglucosamine by islets [23, 24]; the site of this effect is believed to be transport into the cell since it has been found that caffeine impairs islet sugar transport [44]. This action may underlie the paradoxical inhibitory action of methylxanthines on N-acetylglucosamine-stimulated release and biosynthesis of insulin [23, 24]. Thirdly the effects of 3-0-methylglucose were studied [24]. 3-0-Methylglucose is transported into islets by the glucose transport system [45] but is not metabolized and does not stimulate insulin release or biosynthesis. Nor, since glucose-transport is non-rate-limiting, does 3-0-methylglucose inhibit glucose utilization or glucose-stimulated insulin release and biosynthesis [24, 26]. However, competition between 3-O-methylglucose and N-acetylglucosamine would be expected to inhibit N-acetylglucosamine and hence, on the substrate-site hypothesis, N-acetylglucosamine-stimulated insulin release. This prediction has been verified [24].

From these observations the following conclusions have been drawn. 1. Stimulation of insulin release and biosynthesis by sugars involves their metabolism by the B-cell. 2. The 'glucoreceptor' is the enzyme or transport system regulating the entry of the stimulating sugar into metabolism. 3. For glucose itself, glucokinase (perhaps together with glucose 6-phosphatase) constitutes the glucoreceptor: the properties of this enzymic step explain the Km, specificity and sensitivity to mannoheptulose of glucose-stimulated insulin release and biosynthesis. For N-acetylglucosamine, however, the glucose-transporter plays this discriminatory role and constitutes the 'N-acetylglucosamine receptor'; inhibition by phloretin, caffeine and 3-0-methylglucose are exerted at this step. These models are summarized in Tables 2(b) and 3(b).

How Is Glucose Metabolism Linked to Ca^{2+}-Movement?

The concentration of free Ca^{2+} in the B-cell cytosol depends on the rates of several processes: 1. Entry into the B-cell down its concentration gradient; 2. Pumping out of the B-cell against a concentration gradient; 3. Uptake into and release from intracellular organelles; 4. Binding to membranes or soluble components of the cell. Evidence has been obtained relating to two possible control events: the first of these concerns the hypothesis that the extent of reduction of pyridine nucleotides in the B-cell may be a key factor linking glucose metabolism to Ca^{2+}-influx. The second concerns the possibility that islet mitochondrial Ca^{2+}-uptake may play a role in the fine regulation of cytosolic Ca^{2+}-concentration.

Islet Cytosolic NADPH/$NADP^+$ Ratio

Several lines of evidence have suggested that the extent of reduction of pyridine nucleotides may be of importance in stimulus-response coupling in the B-cell. An increase in pyridine nucleotide fluorescence in intact islets of Langerhans was observed on stimulation with glucose and such changes have been correlated with rates of insulin release under various conditions [46, 47]. The techniques used did not permit definition of the identity or cellular location of the reduced pyridine nucleotides. Measurements of whole tissue concentrations of pyridine nucleotides in islets exposed to stimulants such as glucose or pyruvate [48–51] or to inhibitors such as menadione [52, 53], NH_4^+ [54], and methylene blue [55] have also shown correlations between rates of insulin release and the whole tissue concentrations of reduced pyridine nucleotides. However such studies cannot give information on the changes in any particular cellular compartment, whereas ratios of NADPH/$NADP^+$ and NADH/NAD^+ are known to differ by several orders of magnitude between cytosol and mitochondria [56]. In order to overcome this problem of compartmentation an approach previously used for liver [56] has been applied to islets. The principle of the method is that measurement of the concentration of substrate AH_2 and product A of a pyridine nucleotide-linked dehydrogenase can be used to calculate the ratio of reduced to oxidised form of the pyridine nucleotide in a particular cellular compartment.

Since for the reaction $AH_2 + NADP^+ \rightleftharpoons A + NAPDH + H^+$

$$\text{at equilibrium, } \frac{[A][H^+][NADPH]}{[AH_2][NADP^+]} = \text{Keq}$$

$$\text{then } \frac{[NADPH]}{[NADP^+]} = \frac{[AH_2]}{[A]} \times \text{Keq} \times \frac{1}{[H^+]}$$

certain conditions must be fulfilled [56], the major ones being that the dehydrogenase is confined essentially to one cellular compartment and catalyses a near-equilibrium reaction. For rat islets, evidence was obtained that the activity of 'malic enzyme' was sufficient to catalyse a near-equilibrium reaction. The maximal extractable activity of 'malic enzyme' was 11 pmol/islet/min at 30° equivalent to 106 μmol/

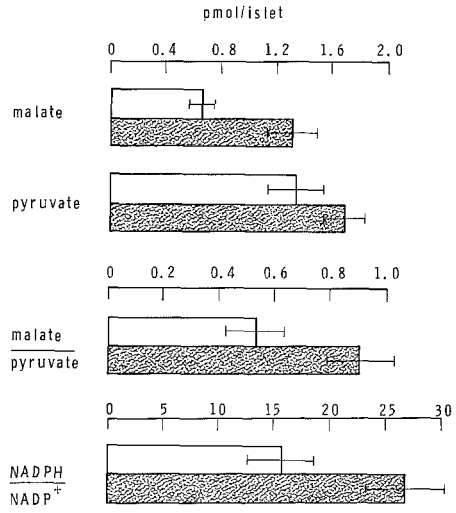


Fig. 2. Effect of glucose on islet cytosolic NADPH/$NADP^+$ ratio. Islets were incubated for 30 min with glucose (2 or 20 mmol/l). The islets were spun through a layer of silicone oil into perchloric acid (PCA) and extracted by heating at 60° for 30 min. Malate and pyruvate in the extract were assayed using malate dehydrogenase and pyruvate dehydrogenase. The NADH formed was measured by bacterial luciferase. The cytosolic NADPH/$NADP^+$ was calculated as described in the text. □ 2 mM glucose, ⊡ 20 mM glucose

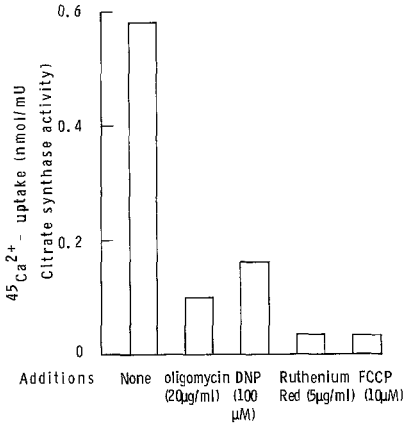


Fig. 3. Effects of uncouplers, oligomycin and ruthenium red on islet mitochondrial $^{45}Ca^{2+}$-uptake. Data from [65]

min/g. The activity of 'malic enzyme' in liver shown to be sufficient to catalyse a near equilibrium reaction is 1.27 µmol/min/g at 25° [56].

Fractionation of islet homogenates showed that 'malic enzyme' was predominantly a cytosolic enzyme. A mitochondrial fraction containing 85% of islet citrate synthase activity contained less than 10% of the 'malic enzyme' activity. Seventy percent of the 'malic enzyme' activity was recovered in a supernatant fraction that contained only 15% of the citrate synthase activity. Hence measurements of islet content of malate and pyruvate could be used to compute islet cytosolic NADPH/$NADP^+$ ratio. Islets were incubated for 30 min with 2 or 20 mmol/l glucose and then rapidly separated from incubation medium by centrifugation through a layer of silicone oil into a quenching solution of PCA. The results are shown in Figure 2: 20 mmol/l glucose increased the islet content of malate significantly but not that of pyruvate. Hence an increase in malate/pyruvate ratio was found. The calculated values of islet cytosolic NADPH/$NADP^+$ ratio were increased from 16 to 26 by 20 mmol/l glucose. It is worth noting that these ratios, as expected, are much higher than the whole tissue ratio of approximately 1 [48–51] thus underlining the necessity for this kind of approach. These findings are consistent with a role for this redox couple in glucose-stimulated insulin release. However the change is less than 2-fold. Other redox couples may show larger changes: the approach used here should permit study of this point. For instance lactate dehydrogenase is likely to catalyse a near-equilibrium reaction: hence measurements of islet lactate/pyruvate ratio will give information on cytosolic NADH/NAD^+ ratio.

Changes in NADPH/$NADP^+$ and NADH/NAD^+ ratio generated by changes in rates of glucose metabolism could be linked to Ca^{2+}-influx in various ways. One attractive possibility is that they may influence, either directly or via some intermediary redox couple such as the glutathione system [57], the extent of reduction of SH-groups in membrane proteins [58] that may determine the cell membrane permeability to K^+. Electrophysiological data and measurements of K^+ or Rb^+ fluxes [59–63] have provided good evidence that Ca^{2+}-influx occurs via a voltage-dependent Ca^{2+}-channel opened by a decrease in membrane permeability to K^+.

Mitochondrial Ca^{2+}-Uptake

In addition to influx into the cell, the rate at which Ca^{2+} is taken up into intracellular organelles is also likely to influence cytosolic Ca^{2+} concentration. Islet mitochondrial Ca^{2+}-uptake may be of particular importance [64] and some properties have been defined [65]. Uptake is dependent on the presence of a respiratory substrate and a permeant anion and is enhanced by $ATPMg^{2+}$. Uptake is inhibited by respiratory chain inhibitors, by uncoupling agents and by ruthenium red (Fig. 3). Uptake of Ca^{2+} is also depressed by methylxanthines and cAMP could not reproduce this effect [6]. Possible links with glucose metabolism were sought by examining the effects of various glycolytic intermediates: inhibition of

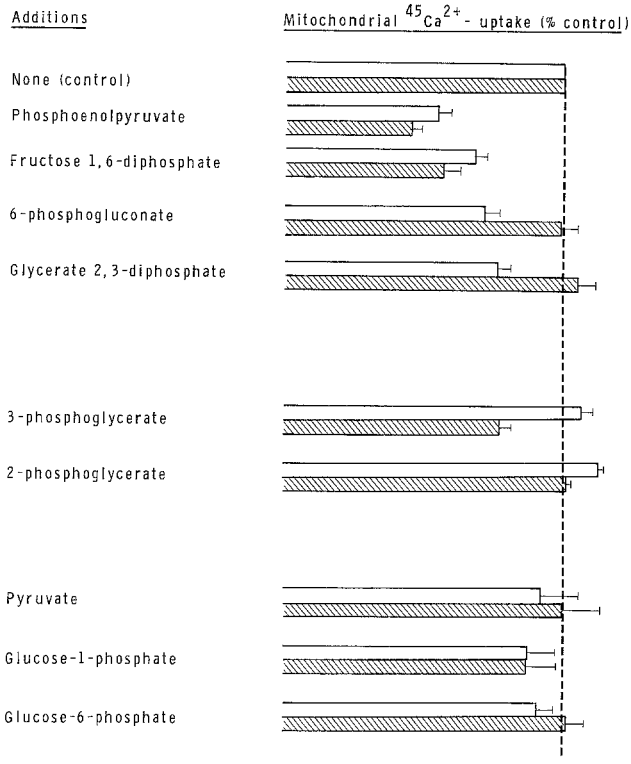


Fig. 4. Effects of glucose metabolites on islet mitochondrial $^{45}Ca^{2+}$-uptake. Mitochondria were incubated for 10 min in media containing 5 mmol/l ATP, 1 mmol/l phosphate and either 1 mmol/l $MgCl_2$, 20 μmol/l $CaCl_2$ and 25 μg/ml albumin (open bars), or 5 mmol/l $MgCl_2$, 220 μmol/l $CaCl_2$ and 200 μg/ml albumin (hatched bars). Dotted line represents control values. Data from [65]

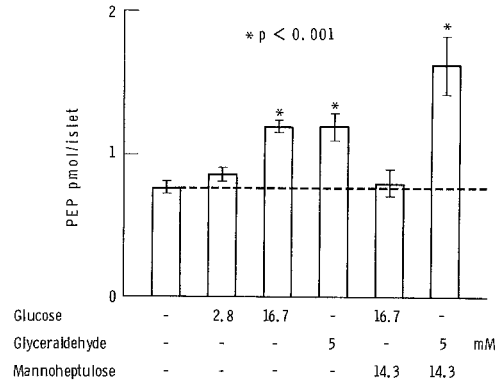


Fig. 5. Effects of glucose, glyceraldehyde and mannoheptulose on the concentration of phosphoenolpyruvate (PEP) in islets. Data from [66]. * $p < 0.001$ compared with control

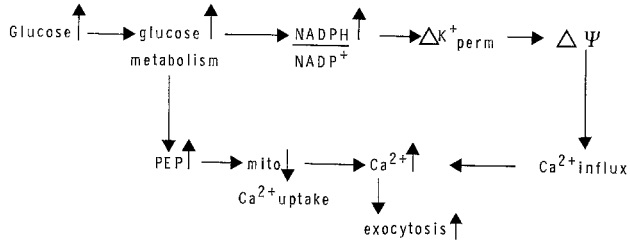


Fig. 6. Metabolic model for stimulus-secretion coupling in the β-cell. For details see text

$^{45}Ca^{2+}$-uptake was observed (Fig. 4) with fructose diphosphate and with phosphoenolpyruvate (PEP). The effects of PEP have been investigated in detail [65]. Inhibition was non-competitive with respect to calcium and was evident over a range of Pi and $ATPMg^{2+}$ concentrations. Moreover [66] the islet content of PEP could be correlated with rates of insulin release when these were varied by glucose or glyceraldehyde, alone or in combination with mannoheptulose (Fig. 5). These findings have suggested that PEP may influence the extent, duration and location of the increase in cytosolic Ca^{2+} in response to glucose, via inhibition of uptake into mitochondria.

Based on these ideas and observations, the speculative model shown in Figure 6 may be proposed to underlie the effects of glucose on insulin release. An increase in glucose concentration leads via an increased rate of glucose metabolism to increased concentrations of PEP and of reduced pyridine nucleotides. The latter influence membrane permeability to K^+ leading to depolarisation and consequent Ca^{2+}-influx into the B-cell. The resultant increase in cytosolic Ca^{2+} triggers exocytosis and is maintained by the restraining effects of PEP on mitochondrial Ca^{2+}-uptake.

With what Components of the Biosynthetic and Secretory Processes Do Ca^{2+}, cAMP and Other Coupling Factors Interact?

There is at present little information on the actual components of the secretory and biosynthetic machinery of the B-cell. However consideration of other systems suggests possible components likely to be the direct mediators of Ca^{2+} and cAMP action. For cAMP in mammalian cells, the major if not the only receptor is cAMP-dependent protein kinase (cAMP-PrK). The properties of cAMP-PrK in rat pancreatic islets have been studied in detail [67] (Table 4). Using DEAE-cellulose chromatography,

Table 4. Properties of islet cAMP-dependent protein kinase

Ka for cAMP	0.8 μmol/l
Km for ATP	16 μmol/l
Km for histone	0.08 mg/ml
Activation by cAMP	x5.9 (Type I)
	x2.6 (Type II)
$S_{20,W}$ (S)	6.73
Stokes radius (nm)	5.2
Mol. wt.	144,200
Frictional ratio	1.5
Axial ratio	6

Values for the Km_{ATP} and activation by cAMP were obtained using rat islet protein kinase partially purified by DEAE-cellulose chromatography: other values were obtained with islet homogenates. For details see [67]

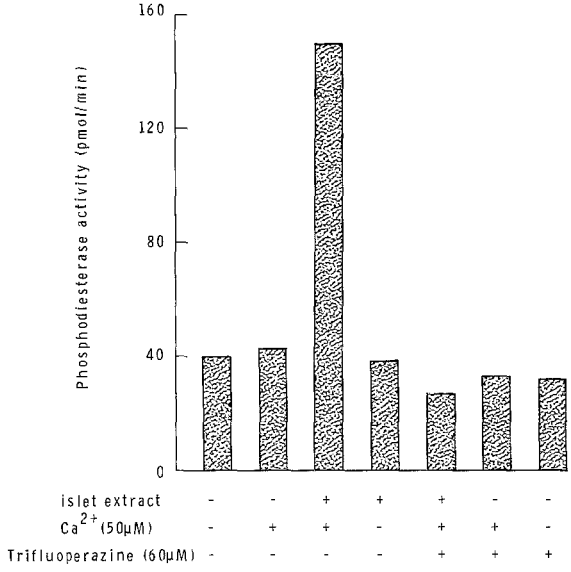


Fig. 7. Activation of phosphodiesterase (PDE) by islet extract: evidence for the presence of calmodulin. The activity of brain PDE was measured in the presence or absence of islet extract, Ca^{2+} and trifluoperazine. For details see [77]

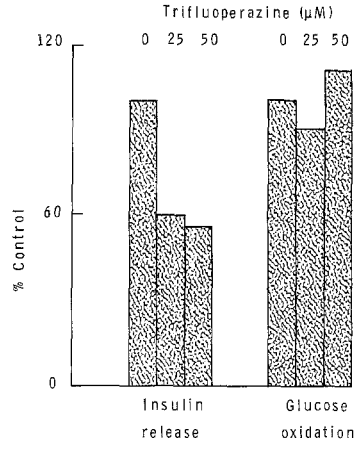


Fig. 8. Effects of trifluoperazine on glucose-stimulated insulin release and islet glucose oxidation. Rates of insulin release and [U-^{14}C] glucose oxidation were measured in islets incubated with 20 mM glucose in the absence or presence of trifluoperazine (25 or 50 μM). For details see [77]

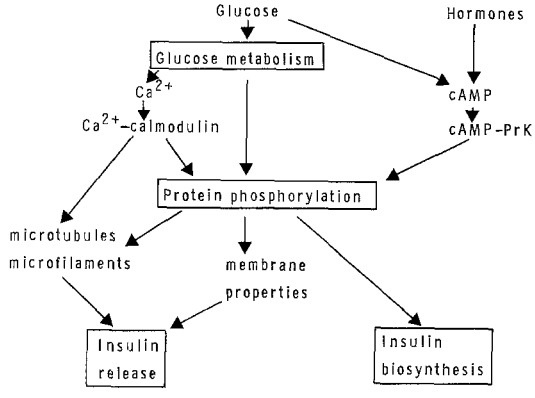


Fig. 9. Possible mechanism in the control of insulin release and biosynthesis. For details see text

it was possible to demonstrate two isoenzymes of cAMP-PrK in islets, corresponding to the Types I and II described in other tissues. Definition of the substrate(s) for these activities seems the most promising approach to understanding the mode of action of cAMP in the B-cell [68].

However these studies also showed that a substantial protein-phosphorylating activity in rat islets was not activated by cAMP nor was it inhibited by a specific protein inhibitor of cAMP-PrK. The nature of this cAMP-independent PrK is not known. However it is tempting to speculate that like certain other cAMP-independent PrK's it may be regulated by Ca^{2+} and of relevance to recent studies which have attempted to define the target for Ca^{2+}-action within the B-cell [77]. A growing body of evidence [69] implicates a family of homologous Ca^{2+}-sensitive proteins in mediating the actions of Ca^{2+} in mammalian cells e. g. troponin C on Ca^{2+}-dependent muscle contraction. Of these Ca^{2+}-dependent proteins, calmodulin (calcium-dependent regulator) may be of particular significance as a general intracellular Ca^{2+}-receptor protein, in view of its ubiquitous tissue distribution and the wide variety of proteins to which it confers sensitivity to Ca^{2+}. Calmodulin was first discovered as a heat-stable activator of brain cyclic nucleotide phosphodiesterase [70]. Later it was found to activate brain adenylyl cyclase [71] and a

Ca^{2+}-ATPase in erythrocyte membranes [72], and to be a component of phosphorylase b kinase [73] and myosin light chain kinase [74] in skeletal muscle. It has also been implicated inter alia in the phosphorylation of various membrane proteins [75] and in the assembly-disassembly of brain microtubules [76]. The hypothesis that calmodulin may play a role in Ca^{2+}-dependent insulin secretion has recently been investigated [77], firstly by demonstrating the presence of calmodulin in extracts of rat islets, and, secondly, by showing that trifluoperazine, a specific inhibitor of calmodulin [78], impairs glucose-stimulated insulin release.

Results demonstrating the presence of calmodulin in rat islets are summarised in Figure 7. Islet extracts were boiled for 3 min and then tested for their ability to activate brain phosphodiesterase. Standard curves of phosphodiesterase-activation were obtained using authentic calmodulin (a gift from Dr. P. Cohen, University of Dundee). Boiled islet extracts activated phosphodiesterase to a maximum of 6-fold, a similar maximum value to that achieved with authentic calmodulin. Activation was dependent on the presence of Ca^{2+} and was abolished by trifluoperazine. The amount of calmodulin in islets, calculated from standard curves, was 1.8 ng/islet. This corresponds to an intraislet concentration of 36 μmol/l, assuming a mean intracellular volume of 3 nl/islet [79] and a molecular weight of calmodulin of 16,700 [73]. The effects of trifluoperazine on intact islets are shown in Figure 8. Insulin release stimulated by 20 mmol/l glucose was inhibited by a low concentration (25 μM) of trifluoperazine that did not affect islet glucose oxidation. This finding is consistent with the hypothesis that calmodulin may play a role in stimulus-secretion coupling in the B-cell. Interpretation of results with 50 μmol/l trifluoperazine is more difficult since although the response to glucose was abolished without impairment of glucose-oxidation, basal insulin release was elevated [77]. Some speculations as to the possible role of calmodulin in B-cell function are put forward in the next section.

General Conclusions and Speculations

Based on the observations and ideas discussed above a tentative working hypothesis for the regulation of insulin release and biosynthesis is shown in Figure 9. The actions of glucose are proposed to be mediated, entirely or in part, by changes in rates of glucose metabolism as discussed above. They involve Ca^{2+} and cAMP primarily concerned with insulin release, and as yet unidentified factors regulating insulin biosynthesis. The intracellular target for Ca^{2+} is suggested to be calmodulin and for cAMP is cAMP-Prk. A major common denominator for these agents is envisaged as protein phosphorylation: protein phosphorylating activity is heterogeneous and comprises cAMP-Prk, mediating effects of cAMP, and cAMP-independent PrK, suggested here to be controlled in part by Ca^{2+}-calmodulin. It is relevant that, in stimulated mast cells, Ca^{2+}-dependent protein phosphorylation has been shown to accompany secretion [80]. Ca^{2+} via calmodulin may also influence the internal cytoskeleton of microtubules-microfilaments directly or by protein phosphorylation. Ca^{2+}-calmodulin may also mediate effects of glucose on cAMP via its effects on adenylyl cyclase and phosphodiesterase. For insulin release it is suggested that phosphorylation of membrane proteins may play a key role in facilitating the membrane-membrane interactions involved in exocytosis. For insulin biosynthesis, it is also conceivable that protein phosphorylation could play a regulatory role, perhaps involving protein kinase activity directly influenced by glucose metabolites. Current studies on the regulation of hemin biosynthesis in reticulocytes [81] provide a precedent for such a model.

Irrespective of whether these speculations prove correct, they have the merit of being susceptible to experimental testing and it is clear that the B-cell will continue to provide a target for extensive investigation. The achievement of a deeper understanding of the control of insulin biosynthesis and secretion remains a fascinating and challenging goal.

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