Glucose-sensing mechanisms in pancreatic β-cells

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The appropriate secretion of insulin from pancreatic β-cells is critically important to the maintenance of energy homeostasis. The β-cells must sense and respond suitably to postprandial increases of blood glucose, and perturbation of glucose-sensing in these cells can lead to hypoglycaemia or hyperglycaemias and ultimately diabetes. Here, we review β-cell glucose-sensing with a particular focus on the regulation of cellular excitability and exocytosis. We examine in turn: (i) the generation of metabolic signalling molecules; (ii) the regulation of β-cell membrane potential; and (iii) insulin granule dynamics and exocytosis. We further discuss the role of well known and putative candidate metabolic signals as regulators of insulin secretion.

Keywords: glucose; insulin secretion; metabolism; ion channels; membrane potential; exocytosis

1. INTRODUCTION

Beta-cells of the pancreatic islets of Langerhans act as glucose sensors, adjusting insulin output to the prevailing blood glucose level. Insulin is critically important for the promotion of glucose storage and the prevention of glycogen breakdown. Thus, the glucose-sensing mechanism of the β-cell is essential for maintenance of glucose homeostasis. This is exemplified by the role that the β-cell plays in the pathogenesis of type 2 diabetes mellitus, as a relative defect in the insulin response to glucose is at least as important as peripheral insulin resistance in the development of the disease (LeRoith 2002; Ashcroft & Rorsman 2004). Deterioration of β-cell function also contributes to the progression of type 2 diabetes (Kahn 2000). As well, several monogenic forms of diabetes are directly linked to defects in the glucose-sensing machinery of the β-cell (Joslin 1978; Mertz 2006). There are three main characteristics of cytosolic glucose metabolism in the β-cell. First, glucose can equilibrate rapidly across the β-cell membrane due to the expression of the high capacity, low affinity glucose transporter-2 (Newgard & McGarry 1995). Second, after glucose has entered the β-cell, it is phosphorylated to glucose-6-phosphate by the high K_M glucokinase (GK, hexokinase IV), which constitutes the flux determining step for glycolysis. Pyruvate, and is suggested to be an important modulator of pyruvate transport or the TCA cycle are not well correlated with glucose-stimulated insulin secretion (Dukes et al. 1997; Zawalich & Zawalich 1997) and inhibitors of pyruvate transport the TCA cycle are well not correlated with glucose-stimulated insulin secretion (Dukes et al.)

Summarize in brief the proximal steps leading to generation of mitochondrial signals and review the effects of these signals on components of the distal glucose-sensing mechanism. Further, we present prospects for additional downstream effects of glucose by speculating on the actions of alternate candidate metabolic signals.

2. PROXIMAL GLUCOSE-SENSING AND METABOLIC SIGNAL GENERATION

(a) Glucose transport and glycolysis

There are three main characteristics of cytosolic glucose metabolism in the β-cell. First, glucose can equilibrate rapidly across the β-cell membrane due to the expression of the high capacity, low affinity glucose transporter-2 (Newgard & McGarry 1995). Second, after glucose has entered the β-cell, it is phosphorylated to glucose-6-phosphate by the high K_M glucokinase (GK, hexokinase IV), which constitutes the flux determining step for glycolysis (Jynedjian 1993; De Vos et al. 1995; Newgard & McGarry 1995; Matschinsky 1996) and is considered as the ‘glucose sensor’ in the pancreatic β-cell (Matschinsky 1996). The fact that mutations in the GK gene lead to impaired insulin secretion in maturity onset diabetes of young type 2 patients further supports a role for GK as a glucose sensor (Porter & Barrett 2005). Third, once phosphorylated, glucose is metabolized by glycolysis to produce pyruvate, NADH and ATP.

The glycolytic product pyruvate is a substrate for the tricarboxylic acid (TCA) cycle in the mitochondria and is suggested to be an important modulator of insulin secretion (Sener et al. 1978; Mertz et al. 1996; Zawalich & Zawalich 1997) and inhibitors of pyruvate transport or the TCA cycle are not well correlated with glucose-stimulated insulin secretion (Dukes et al.).
Ca²⁺ signalling and entry into mitochondrial metabolism lead to the generation of mitochondrial signals. These signals regulate insulin secretion by controlling distal effectors including membrane excitability, Ca²⁺ signalling, insulin granule recruitment and exocytosis. This scheme differs somewhat from the popular concept of ‘triggering’ and ‘amplifying’ pathways, which overlap within the distal mechanism.

1994; Mertz et al. 1996). However, mitochondrial pyruvate metabolism is still likely important in glucose-sensing. Pancreatic β-cells express low levels of lactate dehydrogenase, the enzyme catalysing the conversion of pyruvate to lactate (Sekine et al. 1994; Schuit et al. 1997; Ishihara et al. 1999), and this prevents shunting of pyruvate away from the mitochondrial metabolic pathways (Zhao et al. 2001). Therefore, even though pyruvate provides a substrate to the TCA cycle for ATP production it is not the critical (or at least only) glycolytic factor that initiates insulin secretion. There must be other factor(s) derived from glycolysis, other than pyruvate, required for the generation of mitochondrial signals that lead to insulin secretion. One such candidate factor is NADH (Malaisse et al. 1979; Hedeskov et al. 1987; Pralong et al. 1990; Eto et al. 1999).

(b) Entry into mitochondrial metabolism
Both cytosolic pyruvate and NADH enter the mitochondria and are metabolized to produce ATP (figure 2). The glycolytic factor NADH enters the mitochondria via the glycerol–phosphate and the malate–aspartate shuttle systems (MacDonald 1982; Giroix et al. 1991; Sener & Malaisse 1992; Eto et al. 1999). These shuttles may play an important role in the regulation of insulin secretion (MacDonald 1990). In support of this, glycerol–phosphate shuttle and mitochondrial glycerol-phosphate dehydrogenase (mGPDH) activities are very high in β-cells relative to other mammalian cells (MacDonald 1990). Further support for the model has come from studies of mGPDH −/− mice (Eto et al. 1999). Neither absence of the glycerol–phosphate shuttle (in mGPDH −/− islets) nor suppression of the malate–aspartate shuttle alone (in wild-type islets) altered glucose-stimulated insulin secretion. However, blocking these two pathways together completely ablated the response to glucose, suggesting that both NADH shuttle systems are important for glucose-sensing.

(c) Generation of ATP
In addition to cytosolic NADH, NADH is produced from pyruvate in the mitochondria by the TCA cycle (figure 2). Both cytosolic and mitochondrial sources of NADH stimulate the electron transport chain to pump H⁺ ions out of the mitochondrial matrix, hyperpolarizing the inner mitochondrial membrane (Duchen et al. 1993; Maechler et al. 1997; Kennedy et al. 1998; Eto et al. 1999). The generation of ATP by ATP synthase is coupled to dissipation of the H⁺ gradient. Also, hyperpolarization of the mitochondrial inner membrane can stimulate the mitochondrial membrane potential-dependent Ca²⁺-uniporter to increase mitochondrial Ca²⁺ (Litisky & Pfeiffer 1997), augmenting Ca²⁺-dependent dehydrogenase activity and further increasing the production of NADH and ATP from the TCA cycle (Hansford 1991). A rise in matrix Ca²⁺ may also promote dissociation of an ATP synthase inhibitor, providing a positive feedback mechanism on ATP production (Moreno-Sanchez 1985; Territo et al. 2001). In addition, the increase in mitochondrial Ca²⁺ stimulates ATP transport into the cytosol, increasing cytosolic ATP concentration and the ATP/ADP ratio (Moreno-Sanchez 1985).

Mitochondrial oxidative metabolism has been estimated to produce 98% of β-cell ATP (Erecinska et al. 1992). The exact role of cytosolic or mitochondrial ATP production in the regulation of insulin secretion is controversial. Some investigators have suggested that cytosolic ATP production takes precedence in the regulation of ATP-sensitive channels (Mertz et al. 1996), such as occurring in cardiac muscle (Weiss & Lamp 1989). Support for the important role of mitochondrial ATP production in β-cells is that α-ketoisocaproic acid, which is entirely metabolized in mitochondria by the TCA cycle, can inactivate...
ATP-sensitive K\(^+\) (K\(_{\text{ATP}}\)) channels (Ashcroft & Ashcroft 1990) and stimulate insulin secretion (Best 1997). However, \(\alpha\)-ketoisocaproic acid may block K\(_{\text{ATP}}\) channels by a direct mechanism (Branstrom et al. 1998) and it has been argued that ATP production alone is insufficient for the physiological regulation of insulin secretion and the generation of other mitochondrial signals plays an important role in glucose-sensing (MacDonald et al. 2005). Glutamate may be this mitochondrial factor (Maechler & Wollheim 1999; Maechler et al. 2000; Hoy et al. 2002) although its role in insulin secretion is not currently favoured (MacDonald et al. 2005). Rather, evidence supports an important role for long-chain acyl-CoAs and the mitochondrial export of reducing equivalents (in the form of NADPH) as important distal signals. The generation of these signals, which is summarized below, is the subject of a recent excellent review (MacDonald et al. 2005).

(d) **Long chain acyl CoA**

Prentki, Corkey and co-workers suggested LC-CoA as a potential modulator of insulin secretion (Corkey et al. 1989; Prentki et al. 1992). This model (Deeney et al. 2000b) holds that glucose is in part metabolized to citrate, which is transported out of the mitochondria and converted to malonyl CoA (figure 2). Malonyl CoA then inhibits carnitine palmitoyltransferase 1 (CPT1), a key regulatory enzyme of fatty acid oxidation. Inhibition of CPT1 leads to an increase in cytosolic LC-CoA, the elevation of which could potentiate insulin secretion by direct acylation of regulatory proteins. Alternatively, LC-CoAs may be converted to other bioactive metabolites, for example diacylglycerol (DAG), which activate downstream effectors such as PKC. Correlative experiments have provided support for this model (Corkey et al. 1989; Chen et al. 1994), although it is still controversial since there is no alteration of insulin secretion after the link between glucose and lipid metabolism has been directly perturbed (Antinozzi et al. 1998; Mulder et al. 2001).

(e) **NADPH**

NADPH is another potential mitochondrial signalling molecule derived from glucose metabolism. Metabolizable insulin secretagogues increase the NADPH-to-NADP ratio in rodent islets (Ashcroft & Christie 1979; Hedeskov et al. 1987) and inhibition of NADPH formation reduces glucose-stimulated insulin secretion (Ammon & Steinke 1972; MacDonald et al. 1974). Recently, Newgard’s group (Lu et al. 2002) found, by application of \(^{13}\)C NMR analysis of newly developed INS-1 derived cell lines, that recycling of pyruvate across the mitochondrial inner membrane (figure 2) correlates well with glucose-responsiveness. There are several pathways for pyruvate recycling and NADPH production. One key pathway is the pyruvate–malate shuttle system. The first step of this pathway involves a TCA cycle anaplerotic step where pyruvate is converted to oxaloacetate (OAA) via the pyruvate

**Figure 2. Mitochondrial signal generation.** NADH generated from pyruvate entry into the TCA cycle and NADH transported into the mitochondria by the malate–aspartate (Mal–Asp) and glycerol–phosphate (Gly–P) shuttles drive respiratory chain generation of a H\(^+\) gradient and hyperpolarization of the mitochondrial matrix. H\(^+\) gradient dissipation drives ATP production by ATP synthase. Matrix hyperpolarization promotes the entry of Ca\(^{2+}\) which further stimulates TCA cycle activity and export of ATP. The export of malate and citrate (also isocitrate, not shown) and subsequent conversion to pyruvate generates an increase in cytoplasmic NADPH. Also, exported citrate can be converted to acetyl-CoA and then malonyl-CoA which blocks the LC-CoA transporter carnitine palmitoyltransferase 1 (CPT1). This leads to an increase in cytoplasmic LC-CoA and potential downstream lipid-derived messengers such as diacylglycerol (DAG). Important signals for distal signalling are boxed in grey.

Phil. Trans. R. Soc. B (2005)
carboxylase (PC) reaction. This anaplerotic step is a key step in β-cells since 40–50% of all pyruvate in mitochondria enters the PC reaction (MacDonald 1993). While OAA is part of the TCA cycle, it can be converted to malate by mitochondrial malate dehydrogenase. Malate can participate in numerous pathways including the malate–aspartate shuttle (shuttles NADPH into mitochondria) and the pyruvate–malate shuttle (shuttles NADPH out of the mitochondria). In the latter, malate is transported out of the mitochondria via a malate–Pi antiporter and then converted to pyruvate via malic enzyme resulting in the generation of CO2 and NADPH. Pyruvate can then be transported back into the mitochondria via a pyruvate–H+ symporter, and the cycle continues. Additionally, NADPH reducing equivalents can also be exported from the mitochondria as citrate and isocitrate (MacDonald et al. 2005).

3. DISTAL SENSING OF METABOLIC SIGNALS: MEMBRANE EXCITABILITY

Glucose depolarizes the pancreatic β-cell membrane potential and initiates firing of action potentials (Dean & Matthews 1968, 1970a). It was recognized quite soon that membrane depolarization was dependent on metabolism of the sugar (Dean & Matthews 1970b). In the absence of stimulatory glucose (generally less than 5 mM), rodent β-cells are electrically silent, with a resting membrane potential of approximately −70 mV (Meissner & Schmelz 1974; Matthews & Sakamoto 1975) due to a high resting K+ conductance in these cells (Meissner et al. 1978). Reduction of the resting K+ conductance by stimulatory glucose leads to membrane depolarization and initiation of electrical activity characterized by slow wave depolarization with superimposed bursts of action potentials (Dean & Matthews 1970b; Pace & Price 1972; Meissner & Schmelz 1974; Matthews & Sakamoto 1975). As described below and outlined in figure 3, ATP-sensitive K+ (KATP) channels set the β-cell membrane potential and closure of these leads to depolarization. Membrane depolarization triggers action potential firing and opening of voltage-dependent Ca2+ channels (VDCCs), leading to Ca2+ influx which triggers exocytosis. Action potentials are terminated by the opening of voltage-dependent K+ channels which limit Ca2+ entry and thus insulin release.

(a) ATP-sensitive K+ channels

As early as 1970, a role for ATP generation was acknowledged in the initiation of β-cell electrical activity based on the ability of 2,4-dinitrophenol (an uncoupler of oxidative phosphorylation) to prevent glucose-induced electrical responses in mouse islets (Dean & Matthews 1970b). Glucose was known to induce a reduction in rodent islet K+ permeability (Selhin & Taljedal 1975; Henquin 1978). Ashcroft et al. (1984) and Cook & Hales (1984) demonstrated the existence of K+ channels in rat β-cells that were closed by glucose and ATP, respectively. Subsequently, in mouse β-cells these were shown to be the same channel (Rorsman & Trube 1985), closure of which precedes depolarization-induced Ca2+ influx (Arkhammar et al. 1987). ATP-sensitive K+ channels were first described in guinea pig cardiac muscle (Noma 1983) and are now known to be expressed in numerous tissues including smooth and skeletal muscle, neurons, peripheral axons and epithelial cells (Ashcroft & Ashcroft 1992). The β-cell KATP channel was the first cloned from insulinoma cells in 1995, along with its regulatory sulphonylurea receptor (SUR) subunit (Aguilar-Bryan et al. 1995; Inagaki et al. 1995; Sakura et al. 1995). There are currently two genes known for each of the KATP pore-forming subunits (Kir6.1 and 6.2) and the associated regulatory subunits (SUR1, 2), although diversity is further increased by alternative splicing of the SUR2 gene (Ashcroft & Gribble 1999). KATP channels are composed of four pore forming inward-rectifier K+ channel subunits (Kir6.2 in β-cells; two transmembrane domains) and four regulatory sulphonylurea receptor subunits (SUR1 in β-cells; total 17 transmembrane domains; Aguilar-Bryan & Bryan 1999; Ashcroft & Gribble 1999).

In the absence of glucose KATP channels have a high open probability and mediate an outward K+ flux that holds the membrane potential near the equilibrium potential for K+ (approximately −70 mV; Atwater et al. 1979; Dukes & Philipson 1996; Rorsman 1997). ATP can inhibit KATP channels at the Kir6.2 subunit in

Figure 3. Stimulus–secretion coupling in the pancreatic β-cell. Glucose entry and mitochondrial metabolism increases the intracellular ATP-to-ADP ratio which leads to closure of KATP channels and membrane depolarization. This activates VDCCs, allowing influx of Ca2+ which triggers exocytosis of insulin granules. Kv channels also activate upon depolarization to mediate action potential repolarization, limiting Ca2+ entry and insulin secretion. Established and putative metabolic signalling molecules may regulate insulin secretion at a number of sites. These include the activity of ion channels, release of intracellular Ca2+ stores, mobilization and priming of secretory vesicles, and exocytosis. Here, we have shown the established (green), likely (yellow) and putative (red) regulatory interactions.
the absence of SUR1 (Tucker et al. 1997; John et al. 1998; Mikhailov et al. 1998) and point mutations within Kir6.2 can attenuate the effect of ATP (Tucker et al. 1997; Drain et al. 1998; Tucker et al. 1998; Koster et al. 1999). A similar effect is mediated by the anti-diabetic sulphonylurea drugs through an interaction with SUR1, a member of the ATP-binding cassette super-family (Aguilar-Bryan & Bryan 1999; Philipson & Steiner 1995; Ashfield et al. 1999), stimulating insulin secretion even in the absence of glucose. The SUR1 subunit also binds ATP (both with and without Mg) and MgADP via the presence of two cytoplasmic nucleotide binding domains (Ashcroft 2000). Binding of MgADP to SUR1 is thought to stabilize the channel in the open configuration, and when the ATP-to-ADP ratio increases, MgADP is displaced, and the Kir6.2 subunit becomes available for block by ATP (Ashcroft 2000). A number of excellent reviews focus with more detail on the function and role of KATP channels (Aguilar-Bryan & Bryan 1999; Ashcroft & Gribble 1999; Ashcroft 2000).

Evidence from rat hypothalamic neurons suggests that KATP channels may be regulated independently of ATP (Ainscow et al. 2002); however, the nature of this signal is unknown. One possibility is that KATP activity is modulated by additional metabolically regulated nucleotides. Vascular smooth muscle relaxation following hypoxic pulmonary vasoconstriction is characterized by a time-dependent increase in the NADH-to-NAD ratio and concomitant activation of KATP channels (Shigemori et al. 1996). High micro-molar concentrations NAD(P) and NAD(P)H block KATP channels (Dunne et al. 1988; Harding et al. 1997), likely due to an interaction with the nucleotide inhibitory site of Kir6.2 when SUR1 is co-expressed (Dabrowski et al. 2003). It has been hypothesized that this interaction contributes to the resting level of channel inhibition (Dabrowski et al. 2003); however, a role for this in glucose-sensing is not clear. Since, the NAD(P)-to-NAD(P)H ratio is regulated by glucose it is possible that this plays a physiological role in the modulation of KATP during glucose-sensing.

In addition to regulation by nucleotides, KATP channels are modulated by lipid-derived signals that may play an important physiological role in relation to glucose-sensing. LC-CoA esters activate KATP in mouse (Branstrom et al. 1997) and human β-cells (Branstrom et al. 2004), and also activates the cloned channel (Branstrom et al. 1998b; Fox et al. 2003; Manning Fox et al. 2004). This effect, however, is expected to hyperpolarize the β-cell and thus not contribute to glucose-stimulated secretion per se, but may play an important role in the reduced secretory response observed in type 2 diabetes.

**Voltage-dependent calcium channels**

The ability of pancreatic β-cells to respond to glucose has long been known to depend on extracellular Ca2+ (Curry et al. 1968; Hales & Milner 1968). Action potentials in rodent β-cells result largely from activation of a Ca2+ current rather than a Na+ current (Dean & Matthews 1970b; Meissner & Schmelz 1974; Matthews & Sakamoto 1975). A limited role for Na+ currents in the rodent models was also suggested by the inability of tetrodotoxin to alter glucose-stimulated electrical activity and insulin secretion (Milner & Hales 1968; Meissner & Schmelz 1974), although Na+ currents may contribute to action potentials in human β-cells (Pressel & Misler 1990). The activation of VDCCs in mouse β-cells was first inferred from voltage noise analysis (Atwater et al. 1981). Subsequently, VDCC currents were measured from isolated mouse (Satin & Cook 1985; Rorsman & Trube 1986) and human (Kelly et al. 1991) β-cells. Depolarization of insulinoma and rat pancreatic β-cells leads to opening of L-type VDCCs (Keahey et al. 1989; Horvath et al. 1998; Ligon et al. 1998). A role for other VDCCs in insulin secreting cells (P/Q-type, N-type and T-type) is controversial, although R-type channels have a recently demonstrated role in later stages (or second phase) of insulin secretion (Jing et al. 2005). Antagonism of L-type channels is sufficient to inhibit insulin secretion in humans, rodents, dogs, and from insulinoma cells (Giugliano et al. 1980; Ohmela et al. 1983; Kanatsuna et al. 1985; Ohta et al. 1993). Therefore, L-type Ca2+ channels are considered the effectors of insulin secretion.

VDCC activity in mouse β-cells can be upregulated directly by glucose-derived signals (Smith et al. 1989), the nature of which remain elusive. One possibility is a direct regulation by ATP as L-type channel activity in rat vascular smooth muscle cells is increased by ATP, independent of channel phosphorylation (Yokoshiki et al. 1997). Application of DAG has also been shown to increase Ca2+ channel activity in insulinoma cells (Velasco & Petersen 1989) and palmitate, which presumably stimulates the accumulation of LC-CoAs, enhances Ca2+ channel activity in mouse β-cells (Olofsson et al. 2004). Finally, redox regulation of these channels cannot be ruled out as diphenylene iodonium, a blocker of NAD(P)H oxidase, antagonizes VDCCs in rat type 1 carotid body cells (Wyatt et al. 1994).

Release of intracellular Ca2+ stores is also thought to be involved in regulating insulin secretion. Intracellular Ca2+ stores may be released by the influx of extracellular Ca2+, called Ca2+-induced Ca2+ release (Graves & Hinkle 2003), or by other external signals in order to enhance or prolong insulin secretion rather than trigger it directly. Acetylcholine and cholecystokinin, for example, signal through inositol triphosphate (IP3) to release internal Ca2+ stores and activate protein kinase c (PKC) (Lang 1999), thereby increasing insulin secretion. Interestingly, IP3-receptor mediated Ca2+ release is potentiated by NADH in a reconstituted lipid vesicle system (Kaplin et al. 1996), and in intact PC12 or cerebellar Purkinje cells exposed to hypoxia the metabolic generation of NADH is implicated in release of Ca2+ stores (Kaplin et al. 1996). Also, the skeletal muscle Ca2+ release channel RyR1 contains an oxidoreductase like domain that may bind adenine nucleotides and function as a redox sensor (Baker et al. 2002). NADH inhibited cardiac ryanodine receptor (RyR) activity but activated skeletal RyRs, likely through interaction with the RyR ATP binding site(s) (Zima et al. 2003). Thus, it is interesting to speculate that NAD(P)H (or the cellular redox state) may have an amplifying effect on Ca2+-induced Ca2+ release.
release or second messenger mediated release of Ca$^{2+}$ stores (Hidalgo et al. 2004).

(c) Voltage-dependent $K^+$ channels

We have recently reviewed the role of voltage-dependent $K^+$ channels in pancreatic $\beta$-cell function (MacDonald & Wheeler 2003). These channels open upon membrane depolarization to mediate repolarization during the action potential down-stroke. $K^+$ channel $\alpha$-subunits consist of six transmembrane domains, a voltage sensor (transmembrane domain 4), a pore forming loop and cytosolic C- and N-termini. $K^+$ channels open in response to membrane depolarization and mediate outwardly rectifying $K^+$ currents that act to repolarize action potentials, although different channel homologues exhibit different properties such as voltage-sensitivity and kinetics of activation and inactivation. Functional channels are a homo- or heterotetrameric complex of $\alpha$-subunits of the same family (Kv1.1-4.1). Kv2.1 is the major $\beta$-cell $K^+$ channel isoform (MacDonald et al. 2001, 2002). Cloned $K^+$ channel subunits of the Kv5.1-5.3 channel families do not mediate currents alone, but instead appear to modulate or inhibit other $K^+$ channel families (Hugnot et al. 1996; Salinas et al. 1997a,b; Stocker & Kerschensteiner 1998). Besides regulatory $\alpha$-subunits, $K^+$ channel kinetics can be modulated by large N-terminal domains that mediate fast inactivation, or by the presence of cytoplasmic $\beta$-subunits (Kv$\beta$1-3) that associate with the channel N-terminus (Rettig et al. 1994; Jing et al. 1999).

Numerous studies suggest that $K^+$ channels, including the predominant $\beta$-cell channel Kv2.1, are regulated by mitochondrial signals (Michelakis et al. 2004). While the direct redox modulation of Kv $\alpha$-subunits (particularly cysteine residues) may modify channel properties (Ruppersberg et al. 1991), the regulatory $\beta$-subunits in particular are proposed to act as sensors of intracellular redox potential (McCormack & McCormack 1994; Chouinard et al. 1995) and regulate channels dependent on NADPH-binding (Bahring et al. 2001; Peri et al. 2001). At least one study has demonstrated that mutation of the Kv$\beta$1.1 (Kv$\beta$1.1) oxidoreductase active site attenuated the ability of this subunit to confer fast inactivation to a Kv1.3 channel (Kv1.5; Bahring et al. 2001). Others have shown that mutation of regions putatively involved in NADPH co-factor binding alter the ability of $\beta$-subunits to promote channel surface expression (Peri et al. 2001; Campomanes et al. 2002). Regulatory $\beta$-subunits have also been implicated in the regulation of $K^+$ channels in response to changes in $[O_2]$ (Coppock et al. 2001).

Recent evidence suggests the existence of membrane associated aldehyde oxidoreductase-like enzyme activity in rat islets (Laclau et al. 2001) and a number of oxidoreductase-like Kv$\beta$ subunits (Kv$\beta$1, 2 and 3) are expressed in human and rat islets and INS-1 insulinoma cells (Chouinard et al. 2000). The ability of the aldehyde reductase antagonist diphenylhydantoin to prevent glucose-stimulated insulin secretion from rat islets supports a role for an NADPH-oxidoreductase activity in stimulus-secretion coupling (Kizer et al. 1970; Levin et al. 1972; Laclau et al. 2001). Indeed, $K^+$ channel activity may be regulated by the NADPH-to-NADP ratio (Tipparaju et al. 2005), analogous to $K_{ATP}$ channel regulation by the ATP-to-ADP ratio. We recently described a potent regulation of native Kv2.1 channels in rat $\beta$-cells whereby raising the cytoplasmic NADPH-to-NADP$^+$ ratio caused currents to inactivate quickly and more completely and a leftward shift in the voltage-dependence of steady-state inactivation (MacDonald et al. 2003). Thus, more channels were already inactivated, and therefore unavailable to repolarize action potentials. This has important implications since the metabolic generation of NADPH may reduce the efficacy of $K^+$ channels in repolarizing the $\beta$-cell and therefore contribute to $\beta$-cell electrical excitability in response to glucose, broadening action potentials and increasing calcium influx. This may in part account for the ability of glucose to modify $\beta$-cell electrical responses even when $K_{ATP}$ channels are already closed by sulphonylureas (Henquin 1998; Best 2002).

(d) Additional electrogenic mechanisms

A number of additional factors may control $\beta$-cell electrical activity in addition to those mentioned above. These include other channels such as Cl$^-$ and non-selective cation channels, and electrogenic pumps such as the Na$^+$/K$^+$ ATPase. While the role of these have not been extensively characterized in pancreatic $\beta$-cells, their metabolic regulation may nonetheless yet prove to make important contributions to glucose-sensing. An insulinoma Cl$^-$ current has been shown to be regulated by ATP (Kinard & Satin 1995; Best et al. 1996). This may contribute to $\beta$-cell depolarization in response to glucose (Best 1997; Best et al. 2000). As well, while an early study suggested a limited role for glucose regulation of the Na$^+$/K$^+$ ATPase in $\beta$-cells (Lebrun et al. 1983), more recent work suggests that a glucose-stimulated reduction in the activity of this electrogenic pump, mediated in part by PKC, may contribute to membrane depolarization (Owada et al. 1999). Certainly, other potential contributors will be revealed as we gain a more complete understanding of the control of $\beta$-cell membrane potential.

4. DISTAL SENSING OF METABOLIC SIGNALS: INSULIN GRANULE DYNAMICS AND EXOCYTOSIS

Glucose-stimulated electrical activity in pancreatic $\beta$-cells serves the general purpose of activating VDCCs and delivering the Ca$^{2+}$ stimulus to sites of insulin granule exocytosis. While the membrane potential is exquisitely controlled by glucose, the distribution and exocytotic competence of the insulin granules themselves must be equally well controlled to ensure an appropriate physiological response to glucose. Insulin granules, similar to secretory vesicles in numerous other cell types, exist within the cell in various functional pools (Rorsman & Renstrom 2003). Shown in figure 3, these include an intracellular reserve pool (90%), a morphologically docked pool (ca 10%), and a readily releasable pool (RRP) that is chemically ‘primed’ for release (0.3–2.2%; Rorsman et al. 2000; Bratanova-Tochkova et al. 2002; Olofsson et al. 2002).
The size of the RRP is a major determinant of the magnitude of the initial secretory response. While in the short-term, priming of granules already docked at the membrane may account for refilling of the RRP, ultimately granules must be mobilized from intracellular reserve pools. The initial (or first phase) exocytotic response can be replicated by any stimuli that increases intracellular Ca\(^{2+}\), causing the release of already docked and primed vesicles. Sustained (or second phase) secretion on the other hand, which is dependent on vesicle mobilization and priming, can only be elicited by metabolizable fuel secretagogues (Gembal et al. 1992; Henquin 2000). Thus, glucose-derived signals are important for amplifying and maintaining secretion by promoting the mobilization and priming of insulin granules from reserve pools.

(a) Mobilization of insulin granules
Granules within the cell undergo extensive movement (Somers et al. 1979; Pouli et al. 1998; Barg et al. 2002b; Varadi et al. 2002). Studies employing total internal reflection fluorescence microscopy demonstrated that second phase secretion results largely from the exocytosis of insulin granules newly recruited to the plasma membrane (Ohara-Imaizumi et al. 2002; Ohara-Imaizumi et al. 2004). In rat β-cells, glucose stimulates an increase in the number of insulin granules associated with the plasma membrane (Straub et al. 2004). Insulin granule movement can be stimulated by glucose (Pouli et al. 1998) or ATP (Varadi et al. 2002). Agents that raise cAMP also increase insulin granule movement (Lacy et al. 1975; Hisatomi et al. 1996) and PKC mobilizes catecholamine granules of chromaffin cells into the RRP (Gillis et al. 1996), although PKC may control secretion at a point between vesicle docking with the plasma membrane and exocytosis (Ammala et al. 1994).

Within the cytoplasm vesicle movement likely occurs along microtubules (Orci et al. 1972; Malaisse et al. 1974), mediated by the ATP-dependent motor activity of the conventional kinesin KIF5B (Varadi et al. 2002, 2003). Disruption of the microtubule network impairs granule movement and both the initial exocytotic response and refilling of the RRP (Ivarsson et al. 2004). Inhibitors of 5′-AMP activated kinase (AMPK) enhance insulin secretion (da Silva et al. 2003; Rutter et al. 2003) and a constitutively active AMPK mutant inhibited glucose stimulated granule movement (Tsuboi et al. 2003). AMPK is, therefore, suggested as a negative regulator of granule recruitment and is inhibited by an increased ATP-to-ADP ratio (and reduced AMP). Thus, metabolically generated ATP (or reciprocal changes in ADP and AMP) may be a crucial factor in recruitment of insulin granules from intracellular reserve pools to the plasma membrane.

Near the plasma membrane, insulin granules are transported along the cortical actin network in an ATP-dependent manner, likely by the motor protein myosin 5A (Varadi et al. 2005). By analogy to melanosomes (Hume et al. 2002; Wu et al. 2002), myosin 5A recruitment to the granule may be dependent on small Rab G-proteins such as Rab27a, which are also shown to be important for insulin secretion (Waselle et al. 2003). Actin remodelling stimulated by glucose (mediated by Cdc42 inhibition) is also suggested to be an important pre-requisite for granule recruitment and insulin secretion (Nevins & Thurmond 2003). Thus proteins dependent on GTP/GDP cycling may play an important role in recruitment of vesicles to the plasma membrane. There are conflicting reports as to the role of membrane depolarization and Ca\(^{2+}\) entry through VDCCs in regulating insulin granule traffic (Somers et al. 1979; Hisatomi et al. 1996; Niwa et al. 1998; Pouli et al. 1998). A role for IP\(_3\) mediated release of Ca\(^{2+}\) stores, which may be augmented by NADH (see above), and Ca\(^{2+}\)-dependent phosphorylation of myosin light chain has been proposed (Iida et al. 1997; Niki 1999).

(b) Exocytosis of insulin granules
The machinery regulating exocytosis has been reviewed extensively (Burgoyne & Morgan 2003; Sollner 2003). A comparison of insulin versus neurotransmitter exocytosis is reviewed by Gerber & Sudhof (2002) and β-cell exocytosis has also been reviewed specifically (Bratanova-Tochkova et al. 2002). Fusion of an exocytotic vesicle with the plasma membrane is mediated by soluble NSF attachment protein receptor (SNARE) proteins. These include the plasma membrane proteins syntaxin and SNAP-25 and the vesicular protein VAMP (synaptobrevin). Synaptotagmin is the likely Ca\(^{2+}\) sensor for exocytosis (Meldolesi & Chieregatti 2004), and in β-cells has been variously reported to be represented by the synaptotagmin III, V, VII, VIII and IX (Mizuta et al. 1997; Brown et al. 2000; Gao et al. 2000; Gut et al. 2001; Iezzi et al. 2004). Various SNARE proteins interact with the VDCCs (Catterall 1999), and this mediates a tight coupling of Ca\(^{2+}\) entry and exocytosis of vesicles in the RRP (Barg et al. 2002a).

Prior to Ca\(^{2+}\)-triggered and SNARE-mediated exocytosis, a docked insulin granule must first be primed for release. That is, certain chemical modifications that are not yet completely defined are required to render a granule competent for release. ATP is essential for granule priming prior to exocytosis (Eliasson et al. 1997). The role of ATP may, however, be limited to a permissive one and ADP seems to be a more dynamic regulator of exocytosis at this distal step (Olsen et al. 2003). The ultimate effector of the ADP effect on exocytosis is not entirely clear, but may involve PI4 kinase activation and downstream effects on the Ca\(^{2+}\)-dependent activator protein for secretion (Olsen et al. 2003). Additionally, there is evidence that intra-vesicular acidification by a V-type H\(^{+}\) ATPase plays an important role in insulin granule priming (Barg et al. 2001; Renstrom et al. 2002). While vesicles that were already primed for release were capable of undergoing exocytosis, inhibition of granule acidification prevented refilling of the RRP and subsequent exocytosis (Barg et al. 2001). Thus, metabolically generated changes in the ATP-to-ATP ratio modulate RRP size by regulating steps between vesicle docking with the plasma membrane and the ultimate priming of the granule for release.

Lipid derived LC-CoA signals may also play an important role in regulating RRP size and exocytosis.
Palmitoyl-CoA stimulated secretion from permeabilized insulinoma cells and Ca\(^{2+}\)-dependent exocytosis in mouse β-cells (Deeney et al. 2000a). Also, palmitate was found to increase the initial exocytotic response of mouse β-cells (Olofsson et al. 2004); however, in this study the direct application of palmitoyl-CoA failed to enhance exocytosis. The role of LC-CoAs as direct regulator of the late stages of exocytosis, therefore, remains unclear, although it has been speculated that fatty acylation of exocytotic proteins mediates a LC-CoA effect on exocytosis (Corkey et al. 2000). Consistent with a potential role for acylation in the regulation of exocytosis, palmitoylation of SNAP-23 enhances membrane fusion (Pallavi & Nagaraj 2003) and the protein acylation inhibitor cerulenin attenuates time-dependent potentiation of secretion by glucose (Yamada et al. 2002).

The latter steps of insulin granule priming and filling of the RRP may also be regulated by lipid-derived signals further downstream of LC-CoA, namely the generation of diacylglycerol and activation of PKC. Recruitment of PKC isoforms to the plasma membrane is stimulated by glucose (Zaitsev et al. 1995), and the PKCβ isoform in particular is recruited to insulin granules and appears to be important for exocytosis (Mendez et al. 2003). While PKC translocation likely results from elevated intracellular Ca\(^{2+}\) rather than a direct effect of lipid-derived signals (Deeney et al. 1996; Pinton et al. 2002), lipid-derived signals may still be important for activating or augmenting PKC activity since phorbol esters enhance insulin secretion even when intracellular Ca\(^{2+}\) is raised by direct depolarization with high K\(^+\) (Ravier et al. 1999). A recently identified highly Ca\(^{2+}\)-sensitive pool (HCSP) of granules is increased in size by activation of PKC (Yang & Gillis 2004) and may provide a mechanism for increasing the release competence of granules in the absence of tight coupling to a particular VDCC. PKC activation may also stimulate Ca\(^{2+}\)-independent but GTP-dependent mechanisms of exocytosis in β-cells (Regazzi et al. 1989; Komatsu et al. 1998; Lee et al. 2003). The target(s) by which PKC mediates these effects is unknown. Although SNAP-25 is phosphorylated by PKC in a glucose-dependent manner, this does not correlate with an effect on insulin secretion (Gonelle-Gispert et al. 2002). It is also of interest to note that lipid-derived signals, such as DAG, can interact directly with proteins involved in exocytosis and vesicular cycling including small GTP-binding proteins and Munc13 (Kazanietz et al. 2000). Recently, Ivarsson et al. (2005) have provided evidence suggesting that NADPH may regulate exocytosis directly through the glutaredoxin and thioredoxin proteins.

5. CONCLUSIONS AND FUTURE PERSPECTIVE

Glucose-sensing in the pancreatic β-cell is of critical importance for maintaining energy homeostasis. Despite this central role, we still do not completely understand how the numerous signals generated by mitochondria regulate the important mediators of cellular excitability and exocytosis. The role of ATP, or perhaps more appropriately the ATP-to-ADP ratio, and its effect on K\(_{ATP}\) channels is generally well understood. However, K\(_{ATP}\) inhibition alone cannot account for the complex secretory response of these cells. Perhaps just as important are numerous other metabolically regulated targets, including other ion channels and electrogenic ion pumps, and certainly effects on insulin granule dynamics and exocytosis. Future study of the alternate mitochondrial signals and distal mechanisms with which they interact will be crucial towards a more complete understanding of β-cell glucose-sensing and eventually the development of new and better treatments for type 2 diabetes.

This work was supported by a grant to PR from the Wellcome Trust. JWJ and PEM were supported by fellowships from the Canadian Institutes of Health Research. PEM currently holds the European Foundation for the Study of Diabetes/AstraZeneca Fellowship in Islet Biology and PR is a Royal Society-Wolfson Research Fellow.

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*Phil. Trans. R. Soc. B* (2005)


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Phil. Trans. R. Soc. B (2005)


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Glossary

AMPK: 5'-AMP kinase
CPT1: carnitine palmitoyltransferase 1
DAG: diacylglycerol
GK: glucokinase
IP₃: inositol trisphosphate
Kᵥ: voltage-dependent K⁺
LC-CoA: long-chain acyl-CoA
LDH: lactate dehydrogenase
mGPDH: mitochondrial glycerol-phosphate dehydrogenase
OAA: oxaloacetate
PC: pyruvate carboxylase
PKC: protein kinase C
RRP: readily releasable pool
RyR: ryanodine receptor
SNARE: soluble NSF attachment protein receptor
SUR: sulphonylurea receptor
TCA: tricarboxylic acid
VDCC: voltage-dependent Ca²⁺ channel