Type 2 diabetes mellitus: not quite exciting enough?

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Type 2 diabetes mellitus is a serious metabolic disease that afflicts around 5% of the population in Western societies and over 150 million people worldwide. It is characterized by elevation of the blood glucose concentration, usually presents in middle age, and is exacerbated by obesity. Both genetic and environmental factors contribute to the disease but in the vast majority of cases the aetiology is still not understood. Here we present a novel hypothesis for the aetiology of type 2 diabetes. We postulate that the electrical activity of the insulin-secreting \( \beta \)-cells of the pancreas acts to integrate the genetic and environmental factors that predispose to disease risk. Our hypothesis is supported by a substantial amount of data gathered from a range of different disciplines and makes predictions that can be tested experimentally both \textit{in vitro} and in man.

INTRODUCTION

Natural history of type 2 diabetes

Diabetes mellitus refers to a range of conditions that are all characterized by elevation of the blood glucose level. It may be roughly divided into two principal varieties, type 1 and type 2 diabetes, which have very different aetiologies (1). Type 1 diabetes presents in childhood as an autoimmune attack on the pancreatic \( \beta \)-cells that results in their complete destruction; consequently, the patient must take insulin for the rest of their life (2). It accounts for \(<\)10% of all diabetes and will not be considered further here. Type 2 diabetes is a very different disease that is reaching epidemic proportions in Western societies and is predicted to affect 300 million people worldwide by 2025 (2–4). Impaired glucose tolerance, which precedes diabetes and is a risk factor for the disease, currently affects a further 200 million worldwide. Until recent years, type 2 diabetes was rarely observed in individuals under the age of 50, but increasing numbers of children are now being diagnosed with the disease (5). This probably reflects the growing prevalence of childhood obesity, as type 2 diabetes is exacerbated by obesity and a sedentary lifestyle. Diabetes leads to a reduced life expectancy and quality of life, and a greater risk of heart disease, stroke, peripheral neuropathy, renal disease, blindness and amputation (6). The direct health care costs of the disease are also considerable, and have been estimated at around 5% of the total annual expenditure on health in Western societies.

Both insulin secretion and insulin action are impaired in type-2 diabetes (reviewed in 7,8). Their relative importance has been hotly debated, but it is now recognized that \( \beta \)-cell dysfunction is a key element in the development of the disease (8–10). Abnormalities in insulin secretion precede the onset of type-2 diabetes and may be present even when subjects show normal glucose tolerance (11–14). By the time of diagnosis, insulin secretion is significantly reduced and it continues to diminish inexorably throughout the course of the disease (11). Type 2 diabetes can also occur in the absence of insulin resistance (15) and, conversely, some severe forms of insulin resistance (such as those caused by mutations in the insulin receptor) may not be accompanied by diabetes (16,17). It now appears that insulin resistance only leads to diabetes if combined with a genetically determined propensity to \( \beta \)-cell dysfunction (15,18). In these individuals, however, insulin resistance plays an important role in the development of diabetes by placing an increased demand upon the \( \beta \)-cell that it is unable to match.

Theoretically, the insulin secretory defect could result from either a failure of \( \beta \)-cell function or a reduction in \( \beta \)-cell mass (due to increased apoptosis or reduced \( \beta \)-cell replication). Most quantitative estimates of \( \beta \)-cell density in post-mortem tissue indicate that type-2 diabetics have either no change or \(<30\% \) reduction in \( \beta \)-cell mass (19,20), that is independent of the duration of the disease (21,22). A substantial reduction in \( \beta \)-cell mass is only found in association with amyloidosis, which occurs at later stages of the disease (23,24). In baboons,
Figure 1. The input resistance of the β-cell membrane determines the ease with which electrical activity can be initiated. It is largely determined by the activity of the KATP channel and is low when KATP channels are open and high when they are shut. From Ohm’s Law (V = IR), it is evident that the same magnitude of current (I) will produce a much greater change in membrane potential (ΔV), and thus in insulin secretion, when KATP channels are closed (R is high) than when they are open (R is low). At low glucose (blue trace) the input resistance of the membrane (Ri) is low, so that injection of a small current (I, black trace above) only depolarizes the membrane by a small amount (ΔVli). At high glucose (red trace), the input resistance is higher (5Ri), so that the same current depolarizes the cell by a much larger amount (ΔVli), and may be sufficient to trigger an action potential (dotted line). Potentiators of insulin secretion such as acetylcholine and arginine produce small inward currents (126,127). They are therefore ineffective in the absence of glucose, when the activity of the KATP-channels is high (i.e. R is low), but are able to stimulate electrical activity and insulin secretion at glucose concentrations that shut most KATP channels (i.e. R is high) (125–128). In vivo, blood glucose levels rarely fall below 3–4 mM in man and thus the β-cell input resistance is always relatively high. This explains why acetylcholine, which is released in response to the sight and smell of food, is able stimulate insulin secretion even before blood glucose levels rise. Likewise, the presence of food in the gut releases incretins, such as GLP-1 and GIP, which also initiate insulin secretion prior to elevation of blood glucose. Because arginine and GLP-1 can stimulate insulin secretion even in type 2 diabetes, the input resistance must already be relatively high at fasting blood glucose concentrations. However, the fact that these agents are less effective in diabetes also suggests that, at the same glucose concentration, the input resistance is not as high as that in non-diabetic β-cells.

Mechanism of insulin release

Insulin is stored in secretory vesicles, which are released in response to elevation of intracellular Ca\(^{2+}\). This results principally from Ca\(^{2+}\) influx across the plasma membrane through voltage-gated Ca\(^{2+}\) channels (27). Opening of these channels is triggered by the electrical activity of the cell. Glucose modulates electrical activity principally via metabolically induced changes in the activity of ATP-sensitive K\(^{+}\) (KATP) channels (31). In the absence of glucose (Fig. 2A), these channels are open and the ensuing K\(^{+}\) efflux keeps the membrane hyperpolarised so that voltage-gated Ca\(^{2+}\) channels remain shut. Glucose metabolism closes KATP channels and depolarizes the β-cell (31). In turn, this leads to opening of voltage-gated Ca\(^{2+}\) channels and initiation of electrical activity (Fig. 2B). The mechanism by which KATP channel closure is coupled to glucose metabolism remains unclear, although it is believed to involve changes in the intracellular concentrations of the adenine nucleotides ATP and ADP (32). The KATP channel is also the molecular target for common antidiabetic drugs, such as the sulfonylureas (33), which have been widely used to treat type 2 diabetes for more than half a century (34). Sulfonylureas close these channels independently of glucose metabolism, and thereby elicit electrical activity and insulin secretion (32,35).
Glucose not only initiates β-cell electrical activity; it also modulates action potential frequency, and thereby Ca\(^{2+}\)-influx and insulin secretion. Electrical activity exhibits a characteristic bursting pattern, which consists of slow oscillations in membrane potential between a depolarized plateau, on which Ca\(^{2+}\)-dependent action potentials are superimposed, and a hyperpolarized electrically silent interval (Fig. 2C) (27,28). As glucose is increased, the duration of the plateau phase increases and that of the silent interval decreases so that electrical activity becomes continuous at \( \geq 20 \) mM glucose (36). This produces a graded increase in action potential firing and Ca\(^{2+}\) influx that accounts for the glucose-dependence of insulin secretion (EC\(_{50}\), \( \sim 10–15 \) mM glucose). A complex interaction between K\(_{ATP}\) channels, voltage-gated Ca\(^{2+}\) channels, Ca\(^{2+}\)-activated K\(^+\) channels, Ca\(^{2+}\) pumps and metabolism is responsible for this bursting pattern of β-cell electrical activity and its modulation by glucose (27,37).

The β-cells are electrically coupled to one another, so that the islet functions as an electrical syncytium (38–40). This serves to coordinate electrical activity and insulin secretion across the islet and accounts for the fact that glucose-stimulated insulin secretion from a single islet is pulsatile and mirrors the slow waves of electrical activity (41).

In addition to glucose, insulin secretion is stimulated by amino acids (e.g. arginine), incretins [e.g. glucagon-like peptide 1 (GLP-1)], neurotransmitters (e.g. acetylcholine) and drugs (e.g. sulfonylureas) (27). Some of these agents, like sulfonylureas, can stimulate release in the absence of glucose because they close K\(_{ATP}\) channels, depolarize the β-cell membrane and elicit electrical activity. In contrast, incretins and neurotransmitters are ineffective in the absence of glucose, but are able to amplify insulin secretion produced by an initiator and can even initiate insulin secretion at glucose concentrations just below threshold. Their action is dependent on the ambient level of K\(_{ATP}\) channel activity, which confers their dependence on the glucose concentration (Fig. 1).

**WHY IS ELECTRICAL ACTIVITY USED TO CONTROL INSULIN RELEASE?**

Why is electrical activity used to control insulin release? After all, it would be possible to achieve a graded response to glucose simply by direct metabolic regulation of exocytosis. Indeed, glucose is still able to elicit a graded increase in insulin
secretion when changes in electrical activity are bypassed: for example, by continuous depolarization of the β-cell (42,43). The answer seems to be that electrical activity enables insulin secretion to be switched on and off quickly and precisely. This is particularly important in the case of insulin, as inappropriate release of the hormone can lead to life-threatening hypoglycaemia. Hypoglycaemia is a common complication in diabetic patients taking sulfonylureas, or patients with congenital hyperinsulinaemia, where insulin secretion cannot be switched off rapidly when blood glucose levels fall. The rapid on–off switch of insulin secretion provided by electrical activity also enables insulin secretion to be pulsatile (44), and so avoids down-regulation of insulin receptors in target tissues. This may explain why metabolic regulation of insulin secretion alone is not sufficient to control blood glucose and underscores the importance of electrical activity in insulin release.

DO CHANGES IN ELECTRICAL ACTIVITY UNDERLIE THE IMPAIRED INSULIN SECRETION IN TYPE 2 DIABETES?

β-Cell dysfunction in type 2 diabetes manifests as a reduction in the insulin secretory response not only to glucose, but also to potentiators of insulin release such arginine, hormones, incretins and sulfonylureas (45–48). Any mechanistic explanation of the disease must also account for all these secretory abnormalities. The permissive role of electrical activity in modulating insulin secretion to both initiators and potentiators of release suggests that the abnormalities in hormone release found in type 2 diabetes might be secondary to changes in electrical activity.

The most direct way to test our hypothesis would be to compare electrical activity in non-diabetic and diabetic human β-cells. Unfortunately, such in vitro data are extremely limited, because of the difficulty in obtaining viable islets from type 2 diabetics. However, we recently found that glucose fails to elicit electrical activity, Ca2+ influx and insulin secretion in β-cells and islets isolated from a type 2 diabetic (unpublished data). This is consistent with earlier reports that glucose did not elevate intracellular Ca2+ (49), and that glucose-stimulated insulin secretion was impaired (50), in type 2 diabetic islets.

Clinical studies are also consistent with the idea that β-cell electrical activity may be impaired in type 2 diabetes. The fact that sulfonylureas and glinides, which close KATP channels (32), are effective in the treatment of type 2 diabetes suggests that KATP-channel closure is not complete in diabetes. On the other hand, the finding that arginine and GLP-1 stimulate insulin secretion in type 2 diabetic patients (45–47) argues that even in diabetic β-cells most KATP channels must be closed at stimulatory glucose concentrations. This is because these agents are only effective when the membrane resistance is high (Fig. 1). As neither potentiator is as potent in type 2 diabetics as in healthy individuals (45–47), however, membrane resistance and electrical activity are likely to be reduced. Thus, we hypothesize that glucose intolerance and type 2 diabetes are associated with a lower electrical resistance of the β-cell membrane in the presence of stimulatory glucose concentra-

DIABETES CORRELATES WITH MUTATIONS/ POLYMORPHISMS IN GENES REGULATING ISLET CELL ELECTRICAL ACTIVITY

It is well established that type 2 diabetes is a polygenic disease (7). It has been linked to polymorphisms in many genes, but how these polymorphisms relate to one another, and to the disease phenotype, has not been established. We postulate that the collective action of several common gene variants, and lifestyle factors, combine to produce a small decrease in β-cell electrical activity, and thus a reduction in insulin secretion. We anticipate that the functional consequences of each individual gene variant will be small, so that a single polymorphism, by itself, is unlikely to result in diabetes. However, the cumulative effect of several such polymorphisms will increase disease risk, and in combination with age and/or obesity lead to overt disease. In effect, electrical activity serves as a bottleneck at which the effects of many different genes and lifestyle factors converge.

Impaired ion channels

Polymorphisms in genes encoding ion channels are obvious causative candidates for the reduction in electrical activity that we propose underlies type 2 diabetes. Importantly, a number of such polymorphisms have been identified. One that has received much recent attention is a polymorphism (E23K) in the Kir6.2 subunit of the KATP channel, with a prevalence of 34% in Caucasians (51). Although early studies failed to show a significant association with type 2 diabetes (51,52), meta-analysis and more recent studies using larger sample sizes have revealed that the K allele accounts for between 11 and 15% of the population risk of type 2 diabetes in Caucasians, with an odds ratio of around 1.5 (53–59). When heterologously expressed in mammalian cells, the E23K polymorphism leads to a 2-fold reduction in the ATP sensitivity of the KATP channel (54) and enhanced activation by MgGDP (55,60). It is therefore expected to reduce β-cell electrical activity and insulin secretion. That such small changes in KATP-channel regulation can indeed exert a marked effect on β-cell function is exemplified by the fact that a 4-fold reduction in KATP channel ATP sensitivity causes severe neonatal diabetes in transgenic mice (61). The functional effects of the E23K polymorphism in man are controversial. Some studies report that insulin secretion is impaired (56), whereas others have failed to find a difference in insulin secretion (62,63), but report that the ability of glucose to inhibit glucagon secretion is impaired (63). It is possible that these differences relate to the genetic background of the patients examined, but more studies are clearly needed. Polymorphisms in SUR1 (e.g. S1369A; 59) have also been linked to type 2 diabetes, but as yet no functional consequences of these polymorphisms have been identified.

Overactivity of KATP-channels is not the only mechanism that may be expected to impair electrical activity. Polymorphisms in genes encoding the voltage-gated Ca2+- and K+-channels involved in generating β-cell action potentials that lead to
decreased or increased channel activity, respectively, could also reduce β-cell electrical activity. Indeed, overexpression of the voltage-gated K
+ channel Kv1.5 decreases the secretory response of islets (64), and deletion of the L-type Ca
++-channel in mice results in reduced insulin secretion and glucose intolerance (65). Furthermore, a 7 mV shift in the threshold for L-type Ca
++-channel activation towards more negative membrane potentials enhanced β-cell electrical activity (66), and led to increased serum insulin levels and hypoglycaemia in mice (67). This suggests that a small positive shift in the activation threshold for these channels might increase the threshold for glucose-dependent electrical activity and thus impair insulin secretion. There is also some evidence that voltage-gated Ca
++-channels are influenced by β-cell metabolism (68), so that it is possible that polymorphisms in metabolic genes may modulate electrical activity and insulin release via channels other than the K\textsubscript{ATP} channel. It is of interest that the β-subunit of the voltage-gated calcium channel (CACNB2) lies with the region of chromosome 10p that was linked to permanent neonatal diabetes in a genome-wide linkage analysis of a large family (69). Finally, cytoplasmic Ca
++-influences the activity of small conductance Ca
++-activated K
+ channels, providing a feedback mechanism that links changes in Ca
++-, handling by the β-cell with electrical activity and insulin secretion (70–72). However, with the exception of the K\textsubscript{ATP} channel subunits Kir6.2 and SUR1, the association of polymorphisms in genes encoding β-cell ion channels with type 2 diabetes has not yet been widely studied.

Polymorphisms in genes encoding proteins that regulate ion channel transcription, membrane targeting or activity may also be expected to influence β-cell electrical activity and thereby insulin secretion. In this context it is interesting that another candidate gene within the chromosome 10p locus is PIP5K2A (59), a phosphatidylinositol 4-phosphate 5-kinase type 2, which may be expected to influence the level of PIP2 in the membrane and thereby K\textsubscript{ATP} channel activity (73).

**Impaired metabolic regulation of ion channels**

Because metabolism regulates β-cell electrical activity, polymorphisms in metabolic genes may also be expected to influence the ability of glucose to stimulate electrical activity and insulin secretion. Support for the idea that impaired metabolic regulation of electrical activity occurs in type 2 diabetes comes from a number of rare (1–5%) monogenic forms of diabetes (7). These are collectively referred to as maturity-onset diabetes of the young (MODY) because they present early in life. The first of these to be identified (MODY2) results from inactivating mutations in glucokinase, the high K\textsubscript{m} enzyme that phosphorylates glucose in β-cells and is rate-limiting for glucose metabolism (10) (Fig. 3). All MODY2 patients are heterozygotes: permanent neonatal diabetes results from homozygous mutations (74). Other forms of MODY are due to mutations in genes (e.g. HNF1α, HNF4α, IpF1) encoding a transcriptional network that regulates the expression of several genes critical for glucose sensing (75–80). Studies on knock-out animals have shown the reduced β-cell glycolytic metabolism caused by MODY mutations leads to a decrease in K\textsubscript{ATP} channel closure and electrical activity in response to glucose, which in turn causes less insulin release (81,82). Mutations in mitochondrial DNA (most frequently A3243G in the leucine tRNA gene) cause maternally inherited diabetes, probably by impairing β-cell metabolism and so reducing electrical activity (83–85). These account for a further ~1–2% of diabetic cases.

There is a demonstrable overlap between MODY and multifactorial type 2 diabetes, and mutations in IpF-1 (MODY4) have been associated with type 2 diabetes (86). As yet, there is no evidence that polymorphisms in other MODY genes contribute to type 2 diabetes. It is pertinent, however, that a mutation in the HNF-1α (MODY3) gene accelerates the onset of type 2 diabetes in the Oji–Cree population by 7 years, and is found in 40% of diabetic Oji–Cree patients (87). Likewise, a −30G/A variant in the β-cell promoter of the glucokinase (MODY2) gene has been implicated in impaired glucose tolerance (88). Furthermore, a polymorphism in HNF4α has recently been found to associate with reduced risk of diabetes (59).

Mitochondrial metabolism generates substantially more ATP than glycolysis and the production of mitochondrial ATP is critical for both glucose-dependent insulin secretion and K\textsubscript{ATP} channel closure (85,89). It is therefore pertinent that polymorphisms in genes that regulate mitochondrial ATP production are associated with type 2 diabetes. UCP2 is an uncoupling protein that resides in the inner mitochondrial membrane and uncouples electron transport from ATP synthesis (90) (Fig. 3). Thus it tends to lower cytosolic ATP levels. Deletion of the UCP2 gene in mice enhances islet ATP production and insulin secretion in response to glucose (91). Conversely, overexpression of UCP2 in β-cells attenuates ATP generation and insulin secretion during glucose stimulation (92). This suggests that the level of UCP2 expression may influence insulin secretion in man. Consistent with this idea, a common polymorphism in the UCP2 promoter (−866G/A) causes a 2-fold increase in the risk of type 2 diabetes in obese white Europeans (93). The β-cell transcription factor PAX6 preferentially binds to, and trans activates, the −866A variant in insulin-secreting (INS-1) cells and is expected to increase UCP2 mRNA expression in islet β-cells, thereby reducing ATP levels, electrical activity and insulin release. The frequency of the −866A variant in the European population is 37% (94), suggesting it may make a significant contribution to type 2 diabetes. Recent studies further suggest that the −866A variant is associated with differences in glucose-stimulated insulin secretion in glucose-tolerant human subjects both in vivo and in isolated islets (95).

A common variant in mitochondrial DNA itself (16189) is also associated with type 2 diabetes (96). This variant causes a T to C transition in a region of mtDNA that lies close to control sequences governing replication and transcription. It is associated with increased fasting plasma insulin in several populations, maternal restraint of fetal growth and thinness at birth (97). Consequently, it has been suggested that it may serve as (one of) the gene(s) underlying the ‘thirsty phenotype’ that is thought to predispose to type 2 diabetes (98,99). It is estimated that about 4% of diabetes in the UK diabetic population is attributable to this gene variant. Because its prevalence is higher in Polynesians (93%) and Pima Indians (50%) the 16189 variant may have a substantially larger effect in these populations (100).
Loss-of-function mutations in the transcription factor peroxisome proliferator-activated receptor (PPAR\(\gamma\)) cause severe insulin resistance and type 2 diabetes, but are very rare (101). A polymorphism in this gene (P12A) is found in many ethnic groups. The more common P allele (frequency 85%) is associated with a 1.25-fold increase in diabetes risk and a population risk of 25% (102). Although the less common A12 allele is associated with a reduced risk of diabetes in the general population (103), in diabetics it is associated with decreased \(\beta\)-cell function and increased disease severity (104). In \(\beta\)-cells, PPAR\(\gamma\) enhances the expression (and activity) of a number of genes involved in glucose sensing, including glucokinase (105) and GLUT2 (106). However, it also upregulates UCP2 (107). These data suggest that the A12 allele, which has reduced transcriptional activity (102), may have opposing effects on \(\beta\)-cell metabolism, acting to reduce ATP levels by decreasing glycolytic activity and at the same time tending to enhance ATP levels by decreasing expression of UCP2. Whether or not the overall result is impaired \(\beta\)-cell metabolism (and thus diabetes) will depend on the relative strengths of these effects, and may also be influenced by the presence of other gene variants or environmental factors.

Whatever the underlying mechanism, however, the data support the view that defective metabolic regulation of \(\beta\)-cell electrical activity is involved in type 2 diabetes.

**THE INSIDIOUS INFLUENCE OF AGE AND OBESITY**

Any explanation of type 2 diabetes must account for the fact that disease develops with age and that it is enhanced by obesity. There is evidence that the interaction of such life style factors with genetic ones may also occur at the level of \(\beta\)-cell electrical activity. In mice, for example, ageing causes a reduction in the sensitivity of the \(K_{ATP}\) channel, and thereby electrical activity, to glucose, an effect that appears to be mediated by decreased \(\beta\)-cell metabolism rather than changes in the \(K_{ATP}\) channel itself (81). This may reflect the well-documented decline in mitochondrial function with age, that is believed to result from accumulating mutations in mitochondrial DNA (84,108). A recent in vivo study showed a 40% decline in mitochondrial oxidative and phosphorylation function in muscle between 27 and 70 years (109). In addition, ageing...
reduces insulin sensitivity (8), thereby placing a greater secretory demand on the β-cell. In part, this may also be related to the decline in mitochondrial function (109).

Obesity promotes insulin resistance, which can lead to insulin insufficiency if the secretory capacity of the β-cell is already lower than normal. However, obesity may also reduce insulin secretion via changes in β-cell electrical activity. Obese individuals (110) and type-2 diabetics (111) have higher circulating levels of free fatty acids (FFAs), which are taken up and metabolized by β-cells. Chronic exposure to FFAs leads to the accumulation of long chain acyl CoAs (LC-CoAs) within the β-cell. LC-CoAs both enhance K_{ATP} channel activity and reduce its ATP sensitivity (112,113), thereby reducing glucose-dependent closure of K_{ATP} channels, electrical activity and insulin secretion. K_{ATP} channel activity may also be enhanced by up-regulation of UCP2 in obesity and a consequent decrease in ATP synthesis (91,114,115). This is mediated by FFA stimulation of UCP2 expression (116), probably via PPARγ (107). Mice that lack UCP2 show an enhanced insulin secretory capacity compared with wild-type mice when maintained on a high-fat diet (114).

Changes in β-cell metabolism as a consequence of age and/or obesity will translate into reduced β-cell electrical activity and insulin secretion, not only to glucose but also to incretins and sulfonylureas.

THE YIN AND YANG OF β-CELL ELECTRICAL ACTIVITY

Finally, we point out that diabetes may not only result from mutations that reduce electrical activity. It is also possible for mutations that lead to excessive β-cell electrical activity to cause diabetes, albeit by a different mechanism. Congenital hyperinsulinism (CHI) is associated with constant insulin secretion (8), albeit by a different mechanism. Congenital mutations that lead to excessive β-cell electrical activity. It is also possible for mutations that reduce electrical activity to be present in patients with focal CHI (121). These β-cells (122,123) show an enhanced secretory capacity compared with wild-type mice when maintained on a high-fat diet (114).

Changes in β-cell metabolism as a consequence of age and/or obesity will translate into reduced β-cell electrical activity and insulin secretion, not only to glucose but also to incretins and sulfonylureas.

A MODEL FOR TYPE 2 DIABETES

We propose that individuals at risk of type 2 diabetes carry one or more polymorphisms in ion channel genes, or in genes regulating their activity, membrane targeting or transcription. The functional effect of these gene variants is a small reduction in β-cell electrical activity, which results in decreased Ca^{2+} influx and insulin secretion (Fig. 4). This does not cause diabetes in early life, because glucose homeostasis is tightly controlled and feedback loops ensure that the β-cell secretory output is adjusted. With age, β-cell metabolic function declines, leading to a reduction in glucose sensitivity, electrical activity and insulin secretion. In non-diabetics this does not pose a problem, as the β-cell adjusts its secretory output. However, in individuals who already have reduced β-cell function, the further reduction in electrical activity means that β-cell is no longer able to compensate, so that insulin secretion declines and glucose intolerance, and subsequently overt diabetes, develop. Obesity exacerbates the situation both by causing insulin resistance (increasing the demand on the β-cell) and by further decreasing insulin secretion from the β-cell. This is mediated, at least in part, by a reduction in β-cell electrical activity. The cellular defects culminating in reduced electrical activity and insulin secretion have been analysed by mathematical modelling (125). It seems possible that, once sufficient experimental data have been accumulated, such models may help to determine in silico how combinations of several gene variants, that individually only have minor effects on β-cell metabolism, electrical activity or secretion, can collectively lead to type 2 diabetes.

Predictions

To be of value, a hypothesis should make a number of testable predictions. Our hypothesis predicts that:

- The risk of type 2 diabetes should be increased in individuals who carry disease-promoting polymorphisms in one or more genes. The more polymorphisms an individual carries, the greater the risk of type 2 diabetes and the earlier the onset of the disease.
- These polymorphisms will be concentrated in genes that, directly or indirectly, regulate β-cell electrical activity. This is not limited to genes encoding ion channels but includes genes that regulate ion channel activity (e.g. metabolic genes, kinases), membrane trafficking and expression (e.g. transcription factors).
- Detailed analysis of genes associated with permanent neonatal diabetes, or MODY, will be of value, as is its possible that mutations producing severe functional effects cause neonatal diabetes, whereas polymorphisms having only mild effects enhance susceptibility to type 2 diabetes.
- Glucose will stimulate electrical activity less effectively, if at all, in type 2 diabetic β-cells than in non-diabetic β-cells. Likewise, glucose will cause less elevation of [Ca^{2+}]_{i}.
- Although sulfonylureas, arginine and incretins will still stimulate electrical activity in diabetic β-cells, they will be less effective than in non-diabetic β-cells (at the same glucose concentration).
- The ability of glucose to close K_{ATP} channels and stimulate electrical activity will decline with age in both diabetic and non-diabetic β-cells, as a consequence of increasing
impairment of mitochondrial function. At the same age, diabetes-prone individuals may have a lower mitochondrial function than those not at risk of the disease.

- Obesity will result in a reduced ability of glucose to stimulate electrical activity in β-cells.
- Cells that possess similar glucose-sensing properties to β-cells, such as the GLP-1 producing L-cells and (probably) glucose-sensing neurones in the brain, will also show impaired electrical activity and disturbed hormonal secretion in type 2 diabetes. Similar candidate genes will be involved.

When assessing the effects of gene polymorphisms on diabetes risk, it is important to be aware that large sample sizes will be essential, as gene variants are likely to have small effects on β-cell function. This holds for functional as well as genetic studies. Furthermore, some gene variants will enhance disease risk, while others may reduce it. Indeed, a single gene variant may have both beneficial and deleterious effects on risk, resulting from its expression in different tissues. Such multiplicity of actions may help explain why the aetiology of diabetes has proved so difficult to sort out.

CONCLUDING REMARKS

The data discussed here are consistent with the idea that impaired regulation of β-cell electrical activity explains the defective insulin secretion found in type 2 diabetes. Electrical activity serves as a common conduit through which effects of polymorphisms in genes involved in metabolism, and lifestyle factors such as age and obesity are integrated and translated into changes in insulin release. Changes in electrical activity can account not only for impaired insulin secretion in response to glucose, but also for the reduced response to incretins, neurotransmitters and sulfonylureas that occurs in type 2 diabetes. We hope that our hypothesis will provoke debate and experiment, and provide increased impetus for the study of type 2 diabetic islets. It will stand or fall on the fruits of this research.

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