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Review

Dysfunctional insulin secretion in type 2 diabetes: role of metabolic abnormalities

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Abstract. Insulin secretion is finely tuned to the requirements of tissues by tight coupling to prevailing blood glucose levels. The normal regulation of insulin secretion is coupled to glucose metabolism in the pancreatic B cell, a major but not exclusive signal for secretion being closure of K^+ATP (adenosine triphosphate)-dependent channels in the cell membrane through an increase in cytosolic ATP/adenosine diphosphate. Insulin secretion in type 2 diabetes is abnormal in several respects due to genetic causes but also due to the metabolic environment of the pancreatic B cells. This environment may be particularly important for the deterioration of insulin secretion which occurs with increasing duration of diabetes. Factors in the environment with potential importance include overstimulation, a negative effect of hyperglycemia per se ('glucotoxicity') as well as adverse effects of elevated fatty acids ('lipotoxicity'). Elucidating the mechanisms behind these factors as well as their clinical importance will pave the way for treatment which could preserve B-cell function in type 2 diabetic patients.

Key words. Insulin secretion; type 2 diabetes; hyperglycemia; fatty acids; islets of Langerhans.

Introduction

Insulin secretion is finely tuned to the requirements of tissues such as muscle and liver and responds appropriately to very small changes in blood glucose. The minute-to-minute regulation of insulin secretion is based on an interplay between glucose-derived signals for secretion and the potentiating and inhibitory endocrine and paracrine influences of other nutrients, hormones and neurotransmitters. The acute effects on insulin secretion are supplemented by time-dependent ones, which include regulation of insulin biosynthesis and B-cell replication and neogenesis.

Abnormal insulin secretion is a hallmark of type 2 diabetes, previously termed non-insulin-dependent diabetes mellitus, and is—along with insulin resistance—a recognized cause of the disease [1–3]. The genetics of type 2 diabetes thus include decreased capacity for insulin secretion and/or dysregulation of release to an extent that varies between individual patients. However, insulin release is affected also by metabolic derangements, as evidenced by the beneficial effects of blood glucose normalization on insulin secretion in type 2 diabetes [4–6]. As discussed below, animal studies indicate that other B-cell functions and perhaps B-cell survival can also be negatively affected by hyperglycemia. Moreover, hypertriglyceridemia with elevated fatty acids which characterize obese type 2 diabetic patients could potentially exert negative effects. Furthermore, it

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can be envisaged that abnormal metabolic conditions preceding the development of type 2 diabetes such as insulin resistance in the so-called metabolic syndrome could have negative effects on B cells, effects which could participate in the development of the disease.

The present review will focus on the metabolic factors which, by interfering with B-cell function, could influence the onset and course of type 2 diabetes. Basic features of the regulation of insulin secretion and other B-cell functions will be summarized, as will current knowledge on genetic influences and the possible importance of intrauterine influences. Effects of overstimulation, and of possible toxic effects of hyperglycemia and of hyperlipidemia will then be discussed in more detail.

Normal regulation of insulin secretion

The reader is referred to several reviews for details on this extensive subject [7–10]. A basic property of the B cell is the overriding regulatory role of glucose on secretion, a role that cannot, under normal physiological conditions, be replaced by any hormonal or neuroendocrine influence. Consensus exists that the glucose signal(s) for secretion are coupled to metabolism of the hexose. The production of adenosine triphosphate (ATP) from glucose is crucial in this regard, as is the ratio ATP to adenosine diphosphate (ADP) (see further below). Production of ATP is coupled to mitochondrial metabolism of glucose. The importance of mitochondrial function is underscored by the severe effects on glucose-induced insulin secretion by mutations in mitochondrial DNA (encoding genes important for respiration) in humans [11] or by experimentally induced inhibition of mitochondrial gene transcription [12]. Also, the shuttling to mitochondria of nicotinamide adenine dinucleotide (NADH) formed in the cytoplasm by glycolysis is important for efficient ATP production [13].

The capacity for transport of glucose by GLUT-2 transporter molecules over the cell membrane is normally in excess of the amount of glucose being metabolized in the B cell [7]. Instead, the phosphorylation of glucose to glucose-6-phosphate by the B-cell-specific glucokinase enzyme is a rate-limiting metabolic event which functions as a glucose sensor. Accordingly, the activation curve of this enzyme almost duplicates the dose-response curve for glucose-induced insulin secretion. One principal metabolic signal induced by glucose is a rise in the cytoplasm ATP/ADP ratio [7, 10]. This ratio serves as the major regulator of the ATP-dependent potassium channel (K^+ATP channel) in the cell membrane of B cells. Glucose increases the ATP/ADP ratio and thereby closes the channel. Closure of the K^+ATP channel, in turn, depolarizes the cell membrane, leading to opening of voltage-dependent Ca^{2+} channels, permitting the inflow of Ca^{2+} into the cytoplasm. A rise in $[Ca^{2+}]_{in}$ then serves as a signal, or at least a prerequisite, for insulin secretion (see further below). The importance of this chain of events is highlighted by the inhibitory effect of diazoxide of glucose-induced insulin secretion. This drug inhibits glucose-induced insulin secretion by opening K^+ATP channels, thereby effectively opposing glucose-induced depolarization and insulin release. Other agents, such as the sulfonylurea compounds, exert glucose-like effects on the K^+ATP channel by binding to the channel-associated SUR-1 protein [10].

The effect of glucose on the K^+ATP channel with resulting Ca^{2+} inflow is not the sole mechanism whereby glucose stimulates insulin secretion. During conditions when Ca^{2+} inflow was achieved by other means, such as stimulation by high concentrations of potassium combined with activation by diazoxide of the K^+ATP channel, it could be shown that glucose still stimulated insulin secretion [14]. Also, transgenic mice with nonfunctioning K^+ATP channels develop only mild hyperglycemia and retain some responsiveness to glucose [15]. The metabolic signal(s) for the K^+ATP-independent effect(s) of glucose has so far not been identified [16], but may involve mitochondria metabolites generated by glucose oxidation [17].

The kinetics of the insulin response to an acute elevation of glucose includes a first phase of secretion of less than 10 min duration, followed by a rising second phase. The mechanisms behind phasic insulin release have not been completely elucidated. Phasic secretion probably depends, at least in part, on the existence of different pools of insulin granules within the B cell, a readily releasable pool primed for secretion in the exocytotic pathway being responsible for first phase insulin secretion [18]. Additionally, phasic secretion may be linked to the kinetics of glucose metabolism [19]. Details of the exocytotic process are presently under intense investigation [9].

The in vitro sensitivity to glucose as a stimulator of insulin secretion is amplified in vivo by hormones and neurotransmitters. The hormones gastric inhibitory polypeptide (GIP) and glucagon-like peptide 1 (GLP1) are released from the gastrointestinal tract upon food ingestion and act as 'incretins', i.e. potentiators of insulin secretion to minute increases in blood glucose after food ingestion [20]. Parasympathetic stimulation potentiates insulin secretion due to the binding of acetylcholine to muscarinic receptors in pancreatic islets. Pancreatic islets are richly innervated both by parasympathetic and sympathetic nerves [21]. The mechanisms of potentiation include the activation of B-cell adenylate cyclase-cyclic adenosine monophosphate (cAMP) (GIP and GLP-1) and activation of phospholipase C (acetylcholine). The latter event gives rise to inositoltriphosphate and diacylglycerol. Inosi-

toltriphosphate liberates Ca^{2+} from the endoplasmic reticulum, and diacylglycerol activates protein kinase C [22]. True to their name, the potentiators, although raising second messenger levels in B cells, are inefficient stimulators at low glucose levels in vitro or in vivo, highlighting the overriding need of the body to protect against hypoglycemia. The mechanisms whereby low glucose interferes with the insulin-releasing effect of the potentiators have not been clarified but probably include absence of the glucose-dependent permissive increase in Ca^{2+} inflow through voltage-dependent Ca^{2+} channels.

Activation of α_{2A}- adrenoreceptors as well as binding of somatostatin and galanin to B-cell receptors inhibits insulin secretion. Mechanisms of inhibition include interaction with inhibitory G proteins in the cell membrane, leading, for instance, to inhibition of B-cell adenylate cyclase [23]. Heterotrimeric G proteins can also inhibit secretion at the level of exocytosis, acting by mechanisms so far insufficiently clarified [9]. Such distal effects are involved in the case of α_{2A}- adrenoreceptor stimulation [9, 24].

Regulation of insulin biosynthesis and B-cell mass

Glucose is the principal regulator of insulin biosynthesis. Both transcriptional and translational events mediate a stimulating effect of glucose [2, 25] which requires metabolism of the hexose. Other nutrients, such as leucine, stimulate insulin biosynthesis by mechanisms similar to glucose. Also, cAMP-raising agents can stimulate insulin biosynthesis, acting, at least in part, as potentiators, akin to their effects on insulin secretion.

In the fetus, the development of B cells, their relationship to other islet cells as well as the total B-cell mass is regulated by the balance of a host of transcription factors, including PDX-1 and pax-6 [26–28]. Hormones such as growth hormone and prolactin stimulate B-cell mitogenesis. During extrauterine life the demands on insulin secretion regulate the total B-cell mass. Insulin resistance accompanying obesity and pregnancy is well known to enhance B-cell mass [26–28]. The underlying factors are not completely elucidated but include at least a minimal rise in blood glucose. Hyperglycemia is a powerful stimulus for B-cell hyperplasia, hypertrophy and also neogenesis from duct epithelium. Reciprocally, decreased demands on insulin secretion, for instance after the end of pregnancy, may reduce B-cell mass by apoptosis [29].

Dysregulation of insulin secretion in type 2 diabetes

Type 2 diabetes is characterized by multiple and profound abnormalities in insulin secretion [1–3]. The earliest recognized and perhaps most important one is the reduction in glucose-induced insulin secretion, encompassing in particular first, but also second phase insulin secretion. Selective loss of first phase secretion can upset the orderly inhibition of hepatic glucose production and peripheral uptake of glucose following a meal. In many patients, particularly those with marked hyperglycemia, the insulin response to glucose is totally or almost totally obliterated. Other secretagogues, notably nonnutrients and cAMP-raising hormones such as glucagon and GLP-1, can induce sizable insulin release in type 2 diabetes [1–3]. However, insensitivity to glucose in type 2 diabetes is not restricted to stimulation by glucose per se, but also includes loss of the normal enhancing effect of elevated glucose on insulin responses to potentiators. Hence, at comparable levels of hyperglycemia, the insulin responses to amino acids and to nonnutrient secretagogues are also decreased in type 2 diabetes [1–3, 30]. Disordered pulsatility of insulin secretion is frequently found in type 2 diabetes [2]. Normal pulsatility of insulin secretion could be advantageous in at least two respects: it could render insulin more efficacious, and it could be energy-efficient for the B cell. However, a pathogenetic role for disordered pulsatility has not been established.

An increase in proinsulin both in absolute terms and in relation to insulin is also a feature of type 2 diabetes [2, 30]. Increased levels of proinsulin are theoretically disadvantageous since i) proinsulin has a lesser blood-glucose lowering effect on target tissues, such as muscle and liver, and ii) proinsulin may enhance growth in other tissues such as endothelium, thereby promoting atherogenesis. Again, the clinical importance of these abnormalities is not clear.

It has become increasingly obvious that insulin secretion in type 2 diabetes deteriorates with increasing duration of the disease [31]. The causes of the deterioration have not been established. Deterioration is not explained by autoimmune influences and is not clearly related to chronological age [32]. Amyloid is formed from the B-cell-produced and secreted islet amyloid polypeptide (IAPP). Amyloid has been suggested to participate directly or indirectly in the decline of B-cell function [33]. The formation of amyloid may be genetically influenced and/or be part of the putative negative effects of metabolic abnormalities discussed below.

Genetic factors determining B-cell function

Because insulin secretion in type 2 diabetes is affected by the diabetic state (see further below) in addition to any genetic influence, one can deduce little on genetics from the dysregulated insulin secretion in overtly diabetic subjects [3]. The importance of genetic factors for B-cell function and insulin secretion is, however, clear

from epidemiological and family studies. The precise genetic causes are starting to unravel. Elucidation of the monogenic causes of type 2 diabetes in the autosomally dominant heredity of MODY (maturity onset diabetes of the young) has been instructive in the type of mutations which could be important for deficient insulin secretion [34]. Mutations in the glucokinase gene which decrease gene activity are linked to the MODY 2 variant. These patients usually have mild hyperglycemia and stable B-cell function throughout life, perhaps in part because of protection from overstimulation and glucotoxicity. The cause of MODY 2 was found as a result of a search for candidate genes. In contrast, the finding that mutations of the transcription factors hepatic nuclear factor (HNF)-1α and HNF-4α were the cause of MODY 3 and 1, respectively, were obtained by linkage analyses. In keeping with generalized effects of transcription factors, these mutations can cause multiple deficiencies in B-cell function. In contrast to MODY2, the evolution of MODY 3 and 1 is progressive [34]. This demonstrates the potential for genetic influence behind the deterioration of insulin secretion in other forms of type 2 diabetes.

Since diabetes in MODY patients is monogenic, the genetic influence in these patients is the easiest to characterize among type 2 diabetic patients. Genetic causes for the remaining $>95\%$ of patients are bound to be polygenic. If a polygenic background includes, but is not limited to, polymorphisms in transcription factors, then the spectrum of genetic influences on insulin secretion in a population of type 2 diabetes patients could be bewilderingly large.

Intrauterine effects on B-cell function

A low birth weight is a recognized risk factor for type 2 diabetes as well as for cardiovascular disease [35]. Such an effect appears largely independent of genetic causes [36, 37]. Low insulin secretion brought about by undernutrition has been proposed as a factor [38]; however, the diabetogenic influence of low birth weight in humans seems more clearly associated with insulin resistance [39, 40].

Also, hyperglycemia during pregnancy may negatively affect glucose homeostasis in the offspring [41, 42]. In animal models both insulin secretion and insulin sensitivity may be negatively affected and can be linked to hyperglycemia [43]; however, the conditions for inducing each of these adverse effects appears to be different [44].

Extrauterine effects on B-cell function

This review will not discuss the possible toxic effects generated by autoimmune processes, toxins and pathogens, a topic certainly relevant for a discussion on the causes of type 1 diabetes. Instead, we focus on possible influences by insulin resistance in the nondiabetic and diabetic state, on overstimulation and on the possible adverse influences of hyperglycemia and hyperlipidemia with elevated fatty acids.

Effects of insulin resistance on B-cell function

Insulin resistance increases the demands on B-cell secretory capacity. It leads by several mechanisms (see above) to increased insulin biosynthesis and B-cell replication and neogenesis. The potential for increasing B-cell capacity is age- and species-dependent and is probably also genetically determined in a given individual. The question arises whether long-term increased demands for insulin secretion can negatively affect B cells by overstimulation. Results in animal models are equivocal [45, 46], probably reflecting species differences. Epidemiological data in humans give some support for the notion of negative effects. Hence, in Pima Indians the duration of obesity is a risk factor for diabetes and low insulin secretion [47]; such was also the case in a Swedish study [48]. The mechanisms behind negative effects are not clarified, but could be similar to those which are operative during overstimulation by hyperglycemia (see below).

Overstimulation by hyperglycemia

In 1986 Leahy et al. reported that 48 h of marked hyperglycemia in normal rats, achieved by massive glucose infusions, produced almost total insensitivity to glucose when insulin release was subsequently measured from perfused pancreas [49]. The desensitizing effect was specific for glucose, other secretagogues, such as arginine, exerting normal or even exaggerated responses. Desensitization was reversible within 24 h. This desensitization was in contrast to the in vivo situation, during which insulin responses to glucose were enhanced rather than decreased [50]. The discrepancy between in vivo and in vitro results may be secondary to stimuli present in vivo but not in vitro, acting in concert with glucose to enhance insulin secretion. In particular, long-term hyperglycemia is found to enhance parasympathetic and decrease sympathetic nervous activity, and this change could provide more stimulation of B-cell secretion.

Sako and Grill later showed that if glucose-induced insulin secretion during the glucose infusion was blocked by the simultaneous infusion of diazoxide, then no desensitization occurred; if anything, insulin responses to glucose were enhanced [51]. Since the levels of hyperglycemia were kept the same in experiments with or without diazoxide, it was concluded that the

desensitizing effect was due to overstimulation rather than to any effects of hyperglycemia per se.

The desensitizing effect of overstimulation was studied further in vitro [52]. It was found to be induced in vitro by only a few hours of glucose stimulation. The decline of glucose-induced insulin secretion during prolonged stimulation in vitro mimicked that first described as the third phase of insulin secretion [53]. The decline of glucose-induced insulin secretion was proportionate to the degree of stimulation and could be totally protected against by blocking glucose-induced insulin secretion by diazoxide.

Which mechanisms explain desensitization by overstimulation and the protective effects of diazoxide? As mentioned above, diazoxide blocks glucose-induced insulin secretion by opening K^+ATP channels. An effect of overstimulation on channel activity could therefore be envisaged. Our experiments showed, however, that the protective effects of diazoxide were only indirectly tied to the action of the drug on the K^+ATP channel, since the drug was not protective against desensitization induced by stimulation with high concentrations of potassium, which depolarize the cell membrane by mechanisms not involving the K^+ATP channel. Furthermore, the results with diazoxide could be mimicked by somatostatin, which inhibits insulin secretion not by interaction with the channel but by interacting with G proteins in the B-cell membrane (fig. 1).

A common denominator of the desensitized state, both in vivo and in vitro, is a reduction of insulin contents in islets or perfused pancreas. Indeed, insulin depletion has been proposed to explain wholly the decrease in glucose-induced insulin secretion [54], in which case the term 'desensitization' may not be appropriate for the phenomenon. However, several observations indicate that the protective effects of diazoxide are only partly explained by effects on insulin contents. In this context, experiments in rat islets using cooling to block glucose-induced insulin secretion were particularly instructive. Cooling below 30 °C inhibits exocytosis of insulin but only marginally decreases glucose-induced Ca^{2+} inflow during glucose stimulation [55, 56] and does not block the generation of second messengers, such as cAMP [57]. We found that cooling during glucose stimulation, while blocking insulin secretion and upholding islet insulin contents, only partially protected against desensitization. Only after exclusion of Ca^{2+} in the incubation media did cooling completely protect against desensitization (fig. 2). These results support the notion that persistent inflow of Ca^{2+}—and/or cellular events following that inflow, but distinct from exocytosis—is negative for B-cell function and participates in the desensitization due to overstimulation.

Furthermore, in human islets 48 h of tissue culture at a high glucose concentration induces profound alterations in Ca^{2+} fluxes [58]. Prominent abnormalities were (i) absence of a Ca^{2+} response to glucose, (ii) a doubling of the post-culture 'resting' level of cytoplasmic Ca^{2+}, and (iii) reduction of slow (0.2/0.5/min) oscillations of Ca^{2+}. The two last mentioned abnormalities were corrected by diazoxide, which also protected an insulin response to glucose. However, diazoxide did not protect a Ca^{2+} rise in response to glucose. These results indicate that overstimulation has profound effects on Ca^{2+} fluxes which may participate in desensitization; however, at least in human islets, glucose may also exert other effects related to the glucose molecule per se or its metabolites.

Other studies in human islets have shown that culture at high glucose for several days leads to downregulation of insulin biosynthesis, possibly secondary to decreased expression of the relevant transcription factors [59]. Still other studies have reported decreased glucose metabolism [60]. The role of overstimulation vis à vis effects of glucose per se could not, however, be discerned from these experiments.

We have obtained strong evidence that the abnormally increased secretion of proinsulin in relation to insulin in

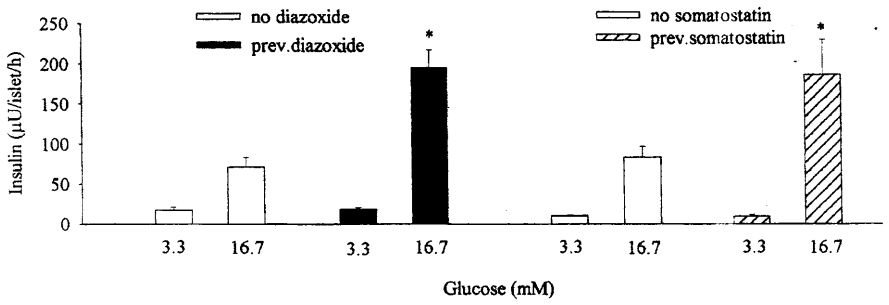

Figure 1. Effects of 20–22-h culture of pancreatic islets from rats in 27 mM glucose ± diazoxide (325 μM) or somatostatin (10,000 ng/ml = 6.1 μM) on glucose-induced insulin secretion in 60-min final batch incubations in 3.3 or 16.7 mM glucose. Data are mean ± SEM of six experiments. * $P < 0.05$ vs. final incubations in 16.7 mM glucose of islets cultured in high glucose only.

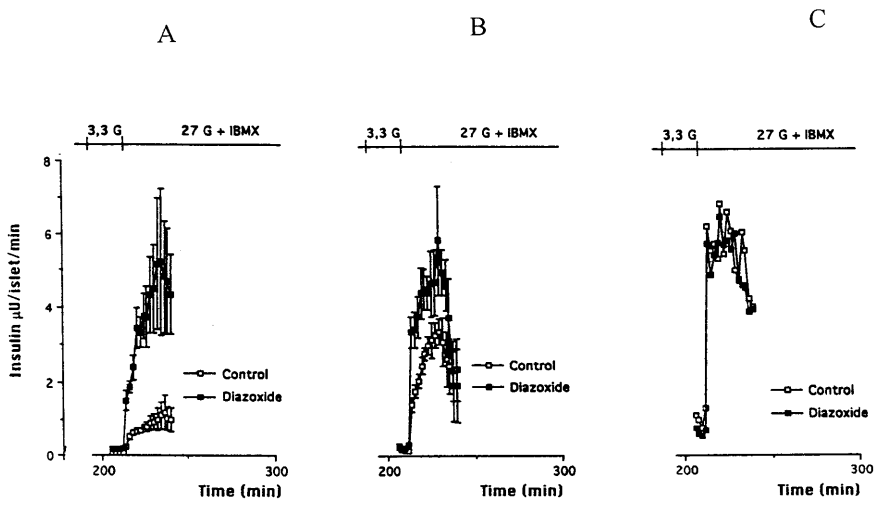

Figure 2. Evidence that a protective effect of diazoxide against desensitization during high glucose exposure is partly linked to reduced Ca^{2+} inflow. Rat pancreatic islets were perifused for 180 min with 27 mM glucose in the presence of 0.2 mM 3-isobutyl-1-methylxanthine (IBMX) in the presence or absence of diazoxide. Islets were then restimulated at the same glucose and IBMX concentration in the absence of diazoxide. In *A* islets were perifused for the 180 min period at 37 °C, in *B* at 22 °C and in *C* also at 22 °C with the omission of added Ca^{2+} to the Krebs-Henseleit-bicarbonate medium. All panels show mean ± SEM of the restimulated responses (which were always performed at 37 °C in the absence of diazoxide). Results are derived from [52]. Reproduced with permission from the Endocrine Society.

type 2 diabetes can be explained in large part by overstimulation. Culture of human pancreatic islets for 48 h at 27 mM glucose thus markedly increased the ratio of immunoreactive proinsulin to insulin in islets and in secreted products both during and after culture [61]. Similar results were obtained in other studies [59, 62, 63]. We additionally find that the increased ratio both of stored and secreted products was normalized by blocking insulin secretion with diazoxide [61]. These results in human islets are analogous to those in rat pancreas of 90% pancreatectomized rats, receiving or not receiving diazoxide [64].

These results do not a priori rule out other influences on proinsulin-to-insulin ratios, such as one of fatty acids [61, see below] or—in relation to type 1 diabetes—an influence of cytokines [65]. Also, although no positive evidence for genetic influences have so far been found, such influences have not been completely ruled out. In this context, it should be noted that a raised proinsulin-to-insulin ratio in plasma in subjects with genetic predisposition for type 2 diabetes need not indicate a primary defect in this parameter, but could instead be a marker of overstimulation of B cells weakened in their capacity by toxic conditions.

The effects of overstimulation so far described were induced by up to 48 h of exposure to elevated glucose in vitro or in vivo. The desensitization obtained under these conditions was readily reversible. The question remains whether chronic overstimulation over months and years would produce irreversible damage to B cells. In support of this notion, we have obtained evidence for a lasting effect of diazoxide treatment on B-cell function in a rat transplantation model [66]. Islets from normal rats were transplanted under the kidney capsule to syngeneic recipients previously made diabetic by streptozotocin. Diazoxide treatment of these rats for 8 weeks improved transplant function not only during, but also after treatment, implying that overstimulation had permanently damaged the transplanted B cells. As to possible mechanisms for such an effect, one should consider the possibility of 'Ca^{2+} toxicity', i.e. the negative effects of continuous inflow of Ca^{2+}, which could possibly activate Ca^{2+}-dependent intracellular proteases, thereby triggering apoptosis [67, 68].

The notion of overstimulation as a negative factor for B-cell function and perhaps survival has obvious clinical implications. Treating type 2 diabetic patients to better control by intensive insulin treatment has repeatedly been shown to improve insulin secretion [4–6]. Also, data from type 1 diabetes indicate long-term beneficial effects on residual insulin secretion by intensive insulin treatment [69] and by diazoxide treatment [70].

Current treatment of type 2 diabetes uses extensively sulfonylurea compounds, which stimulate insulin secretion through a glucose-like effect of the K^+ATP channel. It seems possible that long-term treatment with sulfonylurea could overstimulate B cells with the resulting negative consequences. Clinical studies in type 2 diabetic patients are needed in which these possible

effects of sulfonylurea are examined in patients randomized to either these drugs or to insulin treatment. Such a multicenter study is under way in Sweden, with 2-year results to be reported in the year 2000.

Glucotoxicity

Chronic hyperglycemia over years and decades is a principal cause of the late complications of diabetes, including retinopathy, neuropathy and nephropathy [71]. The mechanisms behind these effects may be multifactorial, involving the generation of advanced glycosylation end products (AGE) and other potentially toxic compounds [62, see further below]. It is natural to pose the question whether hyperglycemia could damage B cells by similar mechanisms. We have tested for effects on B-cell function by aminoguanidine, a guanidine compound which inhibits the formation of AGEs and which retards development of diabetic complications in animal models of diabetes [72]. It was shown that aminoguanidine improved B-cell functions in rat islets cultured at high glucose for 6 weeks [73]. Others have shown that DNA glycosylation interferes with the transcription of the insulin gene [74].

An unresolved question is whether the effects of aminoguanidine can be attributed solely to inhibition of AGE formation, since the compound also has other effects. Aminoguanidine thus inhibits nitric oxide synthase as well as the formation of glucose-derived non-AGE molecules with potential toxicity, for instance methylglyoxal [75]. The possibility that increased oxidative stress associated with hyperglycemia in diabetes could affect B cells should be considered [76], although treatment with vitamin E was basically ineffective in preserving B-cell function in our diabetic transplantation model [77].

Lipotoxicity

It is well known that acute elevation of nonesterified fatty acids (NEFAs) moderately stimulates insulin secretion both at normal and at elevated glucose [78, 79]. Longer-term effects of fatty acids on B-cell function were previously not investigated in any detail. In diabetes, elevated fatty acids may persist for years and even decades. Clearly, long-term effects of fatty acids would be the dominating ones in many obese patients with type 2 diabetes. For these reasons we have performed a series of studies to investigate such effects on B-cell function. The results are summarized below, together with discussion of results of others.

Time dependency of fatty acid effects. Our first study, performed in normal rats [80], established a time dependency for the effects of fatty acids. The rats received a continuous infusion of a fat emulsion, Intralipid, for 3, 6 or 48 h. The Intralipid infusion raised the levels of NEFA threefold. Insulin responses to glucose were measured in the isolated perfused pancreas. A 3-h infusion of Intralipid increased the subsequently tested insulin response to glucose. This stimulatory effect was lost after 6 h of Intralipid infusion. Extending the infusion to 48 h led to a 50% inhibition of glucose-induced insulin secretion.

Mechanisms behind longer-term effects of fatty acids. To investigate the mechanisms behind the inhibitory effect on glucose-induced insulin secretion, we measured glucose oxidation in isolated islets from 48-h Intralipid-infused rats. A high glucose concentration in vitro markedly increased islet glucose oxidation. Previous Intralipid attenuated (by 39%) this stimulatory effect of high glucose [80]. To test whether the inhibitory effect of previous Intralipid was coupled to fatty acid oxidation, we tested for the effects of sodium 2-[6-(4-chlorophenoxy)-hexyl]oxirane-2-carboxylate, etomoxir. This compound inhibits fatty acid oxidation by inhibiting the carnithine-palmitoyl transferase I (CPT-I) enzyme, the activity of which is necessary for the transport of long-chain fatty acid residues into the mitochondria where oxidation takes place [81]. Adding etomoxir in vitro to islets of Intralipid-infused rats significantly ameliorated both glucose oxidation and glucose-induced insulin secretion. These results indicated that the inhibition of glucose metabolism by long-term exposure to elevated fatty acids is linked to fatty acid oxidation.

Tissue culture studies were performed to investigate in more detail how fatty acids induce their inhibitory effects on B-cell function [82]. In vitro, a 24-h period of exposure to fatty acids was needed to document an inhibitory effect on glucose-induced insulin secretion (fig. 3). Inhibitory effects on insulin secretion were accompanied by inhibition of glucose-induced oxidation. In contrast, a moderate stimulatory effect on basal insulin secretion was observed at all times after fatty acid exposure. The inhibitory effects on insulin secretion were reversible within 24 h, indicating that the effects observed were not due to unspecified toxicity.

Observations in other tissues, such as liver and muscle, indicated pyruvate dehydrogenase (PDH) activity as a target for inhibition by fatty acids in a glucose fatty acid cycle [83]. In rat pancreatic islets, a 48-h period of fatty acid exposure in vitro led to a decrease of the percentage of PDH being in the active, unphosphorylated form [84]. Concomitantly, long-term exposure to fatty acids increased PDH kinase activity in islet mitochondria.

The PDH kinase enzyme is known to be strongly associated with the PDH enzyme complex. More recent studies in liver cells have, however, shown that PDH kinase also exists in a non-PDH-bound form [85]. The activity

of 'free' PDH kinase was demonstrated to increase markedly as a result of, for instance, longer-term exposure to fatty acids. Against this background, we measured separately 'free' and PDH-bound PDH kinase from mitochondria of the rat pancreatic islets. Activity measurements after such separation showed that long-term fatty acid exposure preferentially increased the activity of islet 'free' PDH kinase [84].

There is controversy as to the inhibitory effect on PDH enzyme activity, which was found in one other study in rat islets [86] but not in another study employing a glucose-responsive β-cell line [87]. In the latter study there was nevertheless marked inhibition of glucose-induced insulin. It seems likely that neither alterations in PDH and PDH kinase activities nor reduction of glucose oxidation are the only metabolic steps which are important for the negative effects of fatty acids on insulin secretion [88]. An important effect of fatty acids could be uncoupling of mitochondrial metabolism in islets [89], an effect that would render metabolism of glucose less efficient and possibly increase the amount of reactive oxygen species.

Triglyceride accumulation. Long-term elevated NEFA lead to increased islet stores of triglycerides [90, 91]. In this context it is interesting to note that mechanisms for the uptake of lipoproteins exist in human pancreatic β cells [92]. It has been proposed that accumulated triacylglycerols are toxic to B cells, causing cellular depletion and fibrosis [93]. Evidence for this notion was obtained in diabetic Zucker fa/fa rats. Because these rats harbor a leptin receptor mutation [94], they metabolize fatty acids less avidly than normal in pancreatic islets [95]. Consequently, the tendency to accumulate triglycerides in prediabetic and diabetic state is markedly enhanced, the diabetic fa/fa rats accumulating 50–100-fold more triacylglycerols in their B cells than do normal rats [90]. It remains to be demonstrated whether a more moderate increase in B-cell triacylglycerols would also exert toxic effects.

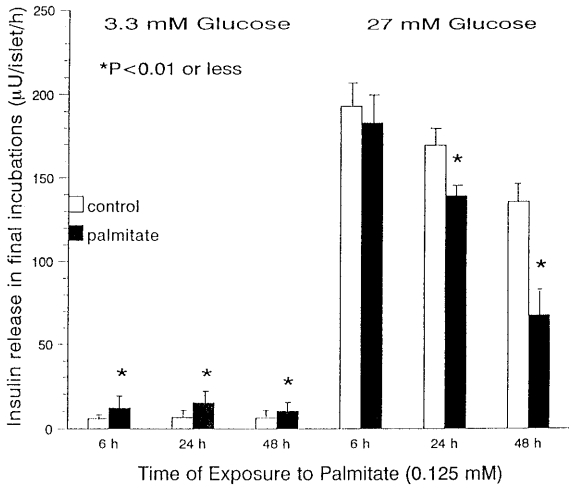

Figure 3. Time dependency of palmitate effects of glucose-induced insulin secretion. Rat pancreatic islets were cultured in RPMI medium containing 11 mM glucose for various time periods with or without the addition of 0.125 mM palmitate. Final incubations after culture were performed for 60 min. Results are mean ± SEM, derived from [82] and reproduced with permission from *Journal of Clinical Investigation*.

Influence of fatty acids on insulin biosynthesis and B-cell replication. Our tissue culture study showed an inhibitory effect on glucose-induced insulin and total protein biosynthesis in rat islets [82], an effect also demonstrable in human pancreatic islets [96]. Such an effect has been confirmed [97]. An effect on insulin biosynthesis may explain the lowered insulin content that was found in fatty acid-exposed islets [82, 96], especially since no signs of intracellular degradation (crinophagy) of insulin could be discerned [97]. In light of the increased demands for insulin biosynthesis during conditions of hyperglycemia and insulin resistance, any inhibitory effect on insulin biosynthesis seems inappropriate and potentially deleterious.

Influence of fatty acids on proinsulin to insulin ratios. As described above, overstimulation exerts a profound influence on the proinsulin-to-insulin ratio of secretion from human pancreatic islets [61]. We also tested the influence of palmitate on this parameter. Culture with palmitate increased the proinsulin-to-insulin ratio in postculture secretion (in the absence of palmitate), but these effects were not correlated to a decrease in the insulin contents of the islets [61]. The effect of the fatty acids on the proinsulin-to-insulin ratio may be due to a delayed processing of proinsulin demonstrated in a pancreatic β-cell line [98]. The molecular mechanisms for such an effect have so far not been elucidated.

In vivo studies. Studies ex vivo or completely in vitro thus document inhibitory effects of long-term fatty acids on insulin secretion. In contrast, discrepant results have been obtained when testing for negative effects in vivo. In rats, inhibitory effects of 48-h infusions with intralipid or oleate decreased in vivo insulin responses to glucose [99]. However, in another study a 48-h Intralipid infusion led to hyperresponsivenes to glucose in vivo [100]. Evidence was presented that the hyperresponse was secondary to fatty acid-induced decreased sympathetic activity.

In nondiabetic humans, a time dependency of the negative NEFA effect could be demonstrated with negative effects on glucose-induced insulin secretion after 24 h of Intralipid infusion [101]. In another study, with 48 h of Intralipid infusion, hyperresponsiveness rather than inhibition of insulin secretion was demonstrated [102]. In a third human study, insulin responses were unaltered after 48 h of Intralipid [103]. Because insulin sensitivity

was concurrently decreased, it was argued that the glucose-induced insulin response during Intralipid should have been increased, an unaltered response reflecting inadequate adjustment of insulin secretion.

From the in vivo studies it thus appears that differences in sympathetic nervous activity and/or fatty acid effects on insulin sensitivity could be important when assessing the discrepant results. In this context one may note that the confounding influence of insulin sensitivity on the assessment of insulin secretion has obtunded the interpretations of normal or abnormal insulin secretion in diabetes for several decades. Indeed, the insulin-resistance effects of chronically elevated fatty acids have no doubt participated in the elevated fasting levels of insulin that are found during both nondiabetic and diabetic conditions. Clearly, this situation makes it difficult to demonstrate negative effects of chronically elevated fatty acids in vivo, especially since criteria for correction for insulin resistance effects can be disputed. The situation with fatty acids is reminiscent of steroid influences: glucocorticoids increase fasting insulin levels and induce B-cell hyperplasia due to insulin resistance; however, in vitro glucocorticoids clearly inhibit insulin secretion [104, 105].

Protocol differences may be also be important for discrepancies between fatty acid studies in vivo. For instance, whereas the study of Paolisso et al. [101] tested insulin response to a sudden rise in glucose at different times during an Intralipid infusion, the Boden study [102] measured insulin secretion during a constantly slightly elevated level of blood glucose. It seems possible that the well-known insulin-stimulating effect of NEFA would persist at basal or only slightly elevated levels of glucose, whereas an inhibitory effect would occur only at higher levels of glucose in vivo.

Elevated NEFA and type 2 diabetes. The db/db mouse is hyperphagic because of a defect in the hypothalamic leptin receptor. When the db gene is present on the.. background the animal develops diabetes early in life [106]. After a 3–6 month period of hyperinsulinemia and adiposity, insulin secretion successively diminishes. The phenotypic traits of the db/db mouse resemble the course of diabetes in obese type 2 diabetic patients. We therefore investigated the influence of elevated NEFA on B-cell function in this animal model [107]. Three-month-old db/db mice were, as expected, obese, hyperglycemic and hyperinsulinemic. Their levels of NEFA were significantly elevated compared with their lean db/+ littermates. Insulin responses to glucose were greatly reduced in islets from db/db mice. Oxidation of C14 was reduced, both in absolute terms and in relation to glucose utilization. Also, the percentage of the active (unphosphorylated) form of PDH was decreased. Exposure to the CPT-I inhibitor etomoxir significantly reversed these abnormalities. However, etomoxir did not achieve normalization. Taken together, these results indicate that long-term elevated NEFA with resulting increased oxidation of NEFA inhibits to a significant extent B-cell function in the db/db mouse and that a decrease in PDH activity is part of this effect.

Others have found that a high-fat diet in diabetic mice inhibits B-cell function [108]. Furthermore, elevated NEFA and increased islet triglyceride stores were found to precede diabetes in fa/fa rats [90]. Available evidence thus indicates negative effects on B-cell function by elevated fatty acids in several animal models of type 2 diabetes.

In human islets long-term elevated NEFA exerted negative effects in addition to those exerted by prolonged exposure to a high glucose concentration [96]. Combined effects of elevated NEFA and hyperglycemia were also observed in a rat model of hyperglycemia through glucose infusions [80]. It is clear from these experiments, as well as from those performed in the db/db mouse, that elevated NEFA is not the only metabolic abnormality that compromises insulin secretion in diabetes.

All the studies referred to have examined the effects of fatty acids ex vivo or in vitro. Definite studies on the influence of fatty acids in vivo in type 2 diabetes patients are lacking. A study reported in abstract form failed to demonstrate any negative effect of 48 h of further elevation of fatty acids in type 2 diabetic patients [109]. Recently, we used the opposite approach and tested the effect of acutely decreasing elevated NEFA in 22 type 2 diabetic subjects by a nicotinic acid derivative, Acipimox. This administration of Acipimox 60 min before a hyperglycemic clamp was accompanied by a modest but significant enhancement of glucose-induced insulin secretion by 20% [110]. These results suggest, but do not prove, that glucose-induced insulin secretion is tonically suppressed by elevated NEFA in human type 2 diabetes.

Concluding remarks

The potential negative influences on B-cell function, growth and survival are clearly multifactorial and may include factors in addition to the ones presently known and discussed. To further elucidate these factors will hopefully open ways to retard by adequate measures the evolution as well as the progression of type 2 diabetes.

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