*For debate***The pathogenesis of NIDDM: the role of the pancreatic beta cell**W.S. Zawalich¹, G.G. Kelley²¹ Yale University School of Nursing, New Haven, Connecticut, USA² Yale University School of Medicine, New Haven, Connecticut, USA

Considerable uncertainty continues to exist regarding the nature of the earliest biochemical changes which culminate in the development of non-insulin-dependent diabetes mellitus (NIDDM) associated with obesity. While a consensus appears to have been achieved concerning the concept that both insulin resistance and defective (or deficient) insulin secretion play important roles in manifest NIDDM [1, 2], less clear is the nature of the initial event which, if uncorrected, leads inexorably to diabetes in so many individuals. Many studies have focused on insulin resistance as the primary defect [3, 4]. In this model of NIDDM genesis, changes at the level of the insulin receptor (altered affinity for insulin, a decrease in their numbers, or a reduction in signalling molecule generation, for example) or postreceptor events (changes in target enzymes and/or their activation) result in impaired sensitivity to insulin's "glucose-regulatory" effects. Insulin resistance at the level of several targets, most notably skeletal muscle, liver, and adipose tissues, results in the inability of insulin to properly direct the disposition of glucose (and other metabolic fuels as well), a more persistent hyperglycaemia and a continued or at least more prolonged stimulation of the pancreatic beta cell. In this model of the development of NIDDM, insulin resistance precedes and results in hyperinsulinaemia. In addition, while the more persistent hyperglycaemia resulting from insulin resistance provides a stronger signal for beta-cell stimulation, the increase in blood

glucose may also, over long periods, exert "toxic" effects on the beta-cell response. Thus, insulin resistance in peripheral tissues requires that the beta cell works harder and for longer periods and if uncorrected may indirectly, via hyperglycaemia, lead to the development of beta-cell decompensation. This adverse effect of hyperglycaemia on beta-cell responsiveness is often referred to as "glucose toxicity" [5].

Consistent with the concept that insulin resistance is the primal manifestation of developing diabetes, or the prediabetic state, are several recently published reports [6–8]. In them, alterations in muscle glycogen synthase (GS) activity have been reported to occur in non-diabetic relatives of NIDDM patients, a population at increased risk for the development of NIDDM, and in obese patients with or without NIDDM. Of particular note for the present perspective is the observation that in the subjects at risk for diabetes, hyperinsulinaemia is already established. Thus, even in studies supporting some type of insulin resistance at the level of target enzymes as the initial alteration, beta-cell hyper-responsiveness is already evident. Is it possible that the hyperinsulinaemia in these patients precipitates insulin resistance at the level of vulnerable insulin-dependent enzymes?

For the last 20 years we have been studying the processes which regulate insulin secretion from pancreatic beta cells. We have been impressed by the ability of cholinergic stimulation to dramatically enhance insulin secretion in the presence of physiologic glucose levels. These responses were observed with exceedingly low levels of cholinergic agonists and resulted in sustained insulin secretory and phosphoinositide (PI) hydrolysis responses. The following is a brief summary of this and other background data. These studies help lay the foundation for the concept that increases in beta-cell secretory activity may be the earliest detectable adaptation, not de-

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Abbreviations: NIDDM, Non-insulin-dependent diabetes mellitus; GS, glycogen synthase; PI, phosphoinositide; GLP-1, glucagon-like peptide-1; GIP, gastric inhibitory polypeptide; CCK, cholecystokinin; CNS, central nervous system; IR, insulin resistance.

fect, which under the appropriate conditions may set into motion the sequence of events with the potential to culminate in insulin resistance and in NIDDM.

Background: the physiologic regulation of insulin secretion

While the stimulatory effect of high glucose levels on beta-cell insulin secretion is well established, the nature of signal transduction systems which mediate this response is still being clarified. Several points are clear, however. First, glucose stimulates insulin secretion via its ability to generate one or more metabolic signals and not via a "glucose receptor" [9–11]. While glycolytic signals may be involved in stimulated insulin release, mitochondrial-generated signals are capable of eliciting a full biphasic insulin secretory response comparable in magnitude to that observed with high glucose alone. Studies with the mitochondrial fuel α -ketoisocaproate, the deamination product of leucine metabolism, fully support this position. Second, and perhaps ironically, over the physiologic range of postprandial glucose levels, glucose by itself has only modest stimulatory effects on insulin secretion [9–12]. However, the insulin stimulatory effect of modest glycaemia is markedly augmented both in vivo and in vitro by the simultaneous presence of the neurotransmitter acetylcholine [13], the neurohumoral agonist cholecystokinin (CCK) [14], and other gut hormones such as glucagon-like peptide-1 (GLP-1) [15] and gastric inhibitory polypeptide (GIP) [14]. In the case of the former two stimulants, their ability to amplify secretion results from receptor-mediated increases in the hydrolysis of membrane PI [16, 17]. On the other hand GLP-1 and GIP exert their effects by increasing cAMP levels in the beta cell [18]. It should be appreciated that the beta cell has multiple inputs. The benefits of the prompt and adequate delivery of insulin to peripheral tissues appears to have favoured the development of complex and, at times, seemingly redundant signalling systems with one main objective: release insulin early and as much as is necessary to insure proper insulinization of target tissues. Our most recent observations with the cholinergic agonist carbachol plus glucose underscore the possible physiologic and pathophysiologic significance of this complex interaction between signalling molecules.

Interactions between glucose and cholinergic agonists. Countless studies have been conducted in an attempt to determine how glucose stimulates secretion. Most investigators, including ourselves, often utilize supra-physiologic glucose levels, usually in the range of 11–28 mmol/l (200–500 mg/dl). Under physiologic circumstances these glucose levels would not usually be anticipated in vivo. However, glucose concentrations

in the physiologic postprandial range, 6–8 mmol/l (108–144 mg/dl) are only modestly stimulatory for insulin secretion. If glucose alone were the sole physiologic stimulant of insulin release, one might anticipate secretory responses insufficient to meet insulin requirements. As previously mentioned, however, an interesting relationship between glucose and a variety of neurohumoral and peptide agonists exist. For example, while the postulated incretin factors CCK or GLP-1 are ineffective stimulants of insulin release in the absence of glucose, in the presence of modest increments of glucose within the physiologic range they (CCK and GLP-1) have a synergistic stimulatory effect on secretion. A similar interaction occurs with cholinergic agonists and glucose and the basis for this synergy deserves some further comment.

Previous investigators have used high glucose plus carbachol combinations to stimulate the beta cell [19]. In general, however, what must be considered supra-physiologic glucose and pharmacologic levels of the cholinergic agonists were employed. In a series of studies with maximally stimulatory glucose (20 mmol/l), we found that this hexose level plus 1 mmol/l carbachol synergistically stimulated both PI hydrolysis and insulin release [20]. In an attempt to define the minimally effective levels of glucose and carbachol at which this interaction occurred, we observed the following. First, levels of carbachol as low as 1 μ mol/l significantly increase PI hydrolysis in 3 H-inositol prelabelled islets. When combined with glucose, synergy (between carbachol and glucose) in terms of PI hydrolysis was observed with glucose levels as low as 3 mmol/l. With physiologic glucose levels (6–8 mmol/l), maximal synergistic effects between glucose and carbachol on PI hydrolysis were observed. When insulin release was stimulated by a moderate physiologic glucose level (8 mmol/l), the addition of 1–10 μ mol/l carbachol results in an amplified and sustained insulin secretory response (Fig. 1). The sensitivity of the beta cell to this agonist combination, which had been observed over 20 years ago [13], suggested to us that any perturbation in terms of stimulant presentation (either glucose or cholinergic agonists) might be accompanied by significant changes in insulin output. This line of reasoning suggests a biochemical basis for the hyperinsulinaemia of obesity and prediabetes.

Studies with models of hyperinsulinaemia and obesity. Because of the dramatic coexistence of obesity and diabetes in many NIDDM patients, a variety of studies have been conducted with animal models to determine the sequence of events which first led to obesity. From the point of view of the present perspective, studies with the obese Zucker rat, central nervous system (CNS)-lesioned animals and, most recently, the sand rat are relevant. In a series of reports Rohner-Jeanraud and coauthors [21–23] have explored

Fig. 1. Acute influence of cholinergic stimulation on beta-cell response patterns. Two groups of freshly isolated rat islets were perfused for 30 min with 5.6 mmol/l glucose (G5.6) and for an additional 30 min with 8 mmol/l glucose (G8) alone (○) or 8 mmol/l glucose plus 10 μ mol/l carbachol (CCh) (●). Insulin secreted into the perfusate was then measured. At least three experiments were performed under each condition and mean values $\pm$ selected SEM are given. Note the dramatic potentiating effect of 10 μ mol/l carbachol in the presence of modest hyperglycaemia. (From Zawalich, W.S., unpublished observations)

the possibility that hyperinsulinaemia, not insulin resistance, may be an early biochemical alteration in this animal model. In studies with preobese fa/fa Zucker rats they found that prior to the development of obesity, at a time when body weights of preobese pups were identical to lean controls, amplified insulin secretory responses from the pancreatic beta cells were evident. Thus, while basal insulin levels in the two groups (preobese and lean controls) were comparable, the secretory responses to both glucose and arginine were increased in the preobese group. Of particular note was the finding that acute pretreatment with the cholinergic antagonist atropine reduced the insulin secretory response. They concluded that increased insulin secretion mediated by the vagus nerve is an early abnormality and that in the adult the hypersecretion of insulin is at least partially vagally mediated. More recent studies using an *in situ* brain-pancreas perfusion model also support this contention; that is, an increase in vagal tone is capable of inducing hyperinsulinaemia [24].

Parallel studies and similar conclusions have been reached using CNS-lesioned animals. For example, ventromedial hypothalamic lesions result in what has been termed hypothalamic obesity. With regards to insulin secretion, it has been found that insulin hypersecretion is one of the earliest (within minutes) detectable alterations [25, 26] and that this beta-cell response can be reversed by atropine. The authors concluded that an increase in vagal tone results in hyper-

insulinaemia. Finally, the result of a series of experiments with the sand rat reinforce the concept that hyperinsulinaemia, in this case induced by nutritional alterations, is the initial biochemical alteration in the development of obesity and diabetes [27]. The authors demonstrated that the sand rat, when placed on an *ad libitum* rodent diet, sequentially develops hyperinsulinaemia, insulin resistance, and finally hyperglycaemia. These changes were fully reversible with the return of normoinsulinaemia. Taken in their entirety, these findings with different animal models suggest that changes in the pancreatic beta-cell response characteristics are fully capable of inducing hyperinsulinaemia, insulin resistance, obesity, and in some instances, diabetes. In the case of the sand rat, the sequence of biochemical changes which occur during their transition from normoglycaemia and normoinsulinaemia to hyperinsulinaemia and hyperglycaemia bear a remarkable similarity to human obesity and diabetes. The fact that nutritional modification precipitates this transition is intriguing. Might a similar sequence of events and similar process occur in the odyssey from prediabetes to diabetes in humans?

Does hyperinsulinaemia exist in prediabetic individuals? The response to this question is positive. In populations at risk for developing diabetes (relatives of NIDDM patients), early and unexplained hyperinsulinaemia has been observed [6, 8, 28, 29]. Both basal and glucose-stimulated insulin secretion rates are generally increased above those from control populations. Hyperinsulinaemia has also been observed prior to the development of diabetes in Pima Indians [30, 31]. Interestingly, beta-cell hyperresponsiveness is also an early event in the development of diabetes in monkeys [32]. Thus, the evidence suggests that hyperinsulinaemia due to enhanced beta-cell responsiveness is an early occurrence in prediabetic and diabetic patients. Our working hypothesis is that events at the level of the beta cell are the earliest changes occurring in the prediabetic individual. Enhanced beta-cell responsiveness, a result of increases in vagal and fuel stimulation of the islet, results in hyperinsulinaemia. Chronic or at least longer-lived hyperinsulinaemia is then responsible for changes in target tissue sensitivity to the glucose-regulatory effect of insulin. Consistent with this concept are findings *in vivo* demonstrating that insulin infusion, in normal subjects which results in modest increments in plasma insulin levels, precipitate peripheral insulin resistance [33–35]. Similar observations have also been made *in vitro*. Most importantly, these changes in insulin action are reversible following the restoration of normoinsulinaemia.

The protean beta cell. The capacity of the pancreatic beta cell to respond to different nutritional situations is remarkable. In times of caloric restriction,

the release of insulin is restrained thus assuring rapid mobilization of stored nutrients. Conversely, in times of excess caloric intake homeostatic mechanisms assure the prompt and quantitatively appropriate release of insulin to meet peripheral tissue requirements for fuel storage and utilization. The complex and sometimes bewildering array of input to the beta cell, while at times seemingly redundant, participates in the physiologic responses of this cell.

Our working hypothesis is that the response of the pancreatic beta cell to increased fuel and/or neurohumoral stimulation usually due to increased caloric intake is the earliest metabolic adaptation in the progression from normal glucose tolerance to obesity to diabetes. While initially this adaptive beta-cell response ensures that the release of insulin will be commensurate to meet insulin requirements, any overshoot in beta-cell responsiveness such as an adaptive increase in vagal tone at the level of the beta cell leaves the individuals at risk for hyperinsulinaemia. The ability of cholinergic stimulation to amplify and sustain insulin secretion has been amply supported by *in vivo* and *in vitro* studies [13, 20–26, 36]. As suggested over 20 years ago by Kipnis [37], the insulin resistance (IR) which follows hyperinsulinaemia may simply be a protective adaptation to prevent insulin-induced hypoglycaemia. In other words, under most conditions most IR is an acquired defect induced by hyperinsulinaemia. If this is a reasonably accurate portrayal of events, then hyperinsulinaemia might be expected to lead to insulin resistance and, conversely, reducing the insulin levels to more physiologic values should restore to a large extent the insulin sensitivity of target tissues. Published studies are consistent with this sequence of postulated events, although it does appear that a small component of IR is not readily reversible [4].

Conclusion

In this era of molecular biology, the search for genes which cause diseases has flourished. Nowhere has this search been more intensive than in diabetes. While point mutations in the glucokinase gene appear to be responsible for an uncommon form of diabetes not usually associated with obesity, less certain are the identity of those genes responsible for most forms of diabetes, particularly NIDDM associated with obesity. For the sake of stimulating more research and further debate, we would suggest that the earliest changes in the prediabetic subject occur at the level of the beta cell. It is adaptive, not maladaptive, in nature and its purpose is to anticipate and ensure that insulin secretion is commensurate to meet insulin requirements. To the extent that this adaptive response can be influenced by neurotransmitters, such as acetylcholine, or gut hormones, such as CCK

Fig. 2. Chronic influence of cholinergic stimulation on beta-cell response patterns. Two groups of islets were incubated for 3-1/2 h with 5.6 mmol/l glucose plus 10 μ mol/l carbachol (CCh). They were then perfused for 30 min with 5.6 mmol/l glucose (G5.6) and for an additional 30 min with 8 mmol/l glucose (G8) plus carbachol (—●—) or 8 mmol/l glucose plus 10 μ mol/l carbachol plus 200 μ mol/l tolbutamide (---●---). At least three experiments were performed under each condition and mean values $\pm$ selected SEM are given. Note that prior chronic exposure to CCh suppresses release to a subsequent 8 mmol/l glucose plus carbachol challenge. However, the sulfonylurea tolbutamide is capable of evoking a secretory response under these same conditions from these islets. (From Zawalich, W.S., unpublished observations)

and GIP-1, an additional element of control and sophistication exists. If, however, the processes that regulate beta-cell adaptation to increased caloric intake overestimate insulin requirements, then hyperinsulinaemia is a risk. In the context of NIDDM, particularly the most common forms associated with obesity, hyperinsulinaemia begets IR, an event already confirmed in both *in vitro* and *in vivo* studies. We would agree that some of the ideas presented in this report are speculative. However, they have as their foundation the results of many experimental studies and they take into account the remarkable flexibility of the beta cell. These studies have shown clear and dramatic effects of cholinergic stimulation on pancreatic beta-cell responses to glucose. The insulin stimulatory action of cholinergic stimulation, although markedly glucose dependent, is apparent even under euglycaemic conditions resulting in basal hyperinsulinaemia. The effects of cholinergic stimulation occur at low levels of neurotransmitter and over physiologically-relevant glucose concentrations; the effects of cholinergic stimulation are long lasting and prime the beta cell for eventual stimulation with glucose (or other fuels as well) resulting in an augmented insulin secretory response; the effects of cholinergic activation can have profound effects on circulating insulin levels: and, finally, chronic stimulation with a cholinergic agonist results in beta-cell decompensa-

tion to glucose stimulation (Fig. 2). Incidentally, islets desensitized to glucose stimulation still display secretory responses to the sulfonylurea tolbutamide, a situation reminiscent of that observed in human NIDDM patients (Fig. 2).

If a change in beta-cell chemosensitivity is the earliest event in most NIDDM patients, this would help explain why hyperinsulinaemia predates the disease, why lowering the insulin levels restores insulin sensitivity, and why diabetes flourishes in populations (such as in Micronesia), where overnutrition (and apparently not gene mutations) along with other lifestyle changes is apparently the major precipitating event in disease emergence [38–40]. This concept is also compatible with and may help to explain recent studies with juvenile obese individuals. They are characterized initially by augmented insulin secretory responses prior to the development of insulin resistance [41]. If beta-cell insulin secretory responses regulate the insulin sensitivity of target tissues, then perhaps future studies should focus on how this remarkable cell adapts both acutely and over time to changing fuel and neurotransmitter presentation and, perhaps, to the genes which regulate these responses.

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