REVIEW ARTICLE

Mechanisms of Glucose Stimulated Insulin Secretion in Health and in Diabetes: Some Re-evaluations and Proposals

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That severe diabetes mellitus is a disease of insulin deficiency has never been questioned. In contrast, for the milder forms of the disease, widely varying (and often conflicting) views have been offered in order to explain the pathophysiology of the metabolic abnormality (for a review of this topic, see 1). However, after many swings in opinion, during recent years most workers seem to acknowledge that even the mildest forms of diabetes are accompanied by deficient insulin production in response to the needs of the organism. This, together with the fact that techniques for hormone measurement and organ isolation have been available for the endocrine pancreas for a number of years, has stimulated a huge number of studies on the mechanism of insulin secretion. Indeed, any young scientist with the intention of working in this field will probably get confused when first confronted with the literature, and unless he is totally unconscious or extremely self-confident, will seek some other topic of inquiry, where simpler questions may be given simple answers. For this reason, I shall attempt in this review to offer a simplified version of how the secretion of insulin may be governed by glucose (and glucose alone, for the sake of further simplicity), and which steps in this process may be modified in diabetes mellitus. To seek for the least number of explanations that may account for the largest amount of observations (accepting the evident risk of oversimplifications) has been my leading motive.

Initiation of Insulin Release by Glucose

One characteristic feature of the glucose-induced insulin release is its rapidity. In most sensitive assay

systems, such as *in vivo* experiments with sampling in the portal vein, or *in vitro* using the perfused whole pancreas preparation, *maximal* rates of insulin release may be attained within seconds to a few minutes after initiation of hyperglycemia [2–4]. Thus, the chain of events involved in the recognition by the B-cell of changes in the extracellular concentration of glucose, the transmission of this message to intracellular sites, movement of B-granules to and/or fusion of the granular membrane with the cell membrane, and finally extrusion of the granule to the extracellular space by emiocytosis, must proceed at a very high speed. The cellular unit responsible for recognition of glucose as an initiator of these processes has been investigated actively during recent years. Unfortunately, the bulk of the existing data stems from experiments in which the kinetic peculiarities of insulin secretion were totally ignored. Thus, the well established view that a product of glycolysis in the B-cell may initiate release was based on correlations obtained between various parameters of glucose utilization on one hand and insulin secretion on the other, in islet incubations of 60–120 min duration where the data were collected in a cumulative manner [5–7]. For this and other reasons, several authors have questioned whether insulin release may not be activated by some direct action of glucose, for instance by binding to a cell membrane receptor [8, 9]. The intense discussion on whether a “glucoreceptor” mechanism is the main route of action of glucose on insulin release seems to us premature. Indeed, data from kinetic measurements of several glycolytic products in the islets have been presented as evidence for both absence and presence of significant early changes in their levels after increasing the extracellular glu-

cose concentration [10–14]. While no correlation between changes in insulin secretion rate and levels of glycolytic intermediates were demonstrated, the concentration of a presumptive insulin releasing metabolite may not need to show large variations if its flux is of greater importance for the cellular release organelles; fluxes have not been measured in such experiments. Therefore, until convincing evidence is presented indicating that glucose without entering the B-cell may release insulin (*e.g.* by incubating islets with particle-bound glucose in the absence of free glucose, but in the presence of an ATP-generating system) it will not be possible to decide whether glucose

sponse is usually somewhat greater. Thus, whatever cellular mechanisms are involved in these two phases of insulin release, their affinity to glucose seems to be identical. It therefore seems more logical, in the present state of knowledge, to suggest that the action of glucose on the initiation of insulin release is uniform with time, only one recognition unit being involved. The marked, time-bound changes that are known to occur in the final response, *i.e.* in insulin secretion rate, may be due to the modulation of the signal elicited by glucose in the B-cell. This latter phenomenon will be discussed later in this review.

Once the increase in extracellular glucose con-

Fig. 1. Similarity of the affinities for glucose of the early and late insulin responses. A) Peripheral plasma insulin response to glucose infusion (priming and constant infusion) in healthy subjects. Early response corresponds to the 10 min values, the late one to the 60 min levels. B) Insulin secretion rates from the isolated rat pancreas stimulated by a square-wave pulse of glucose. Early response is at 2 min, the late one at 40 min after having increased the glucose concentration of the perfusate from 80 mg/100 ml to the respective values (data from S. Efendic, E. Cerasi, R. Luft, to be published). C) Insulin secretion rates from perfused isolated mouse islets. Legend as for B, except that early response is at 5 min, and the late one at 30 min. Basal glucose concentration in the perfusate was 50 mg/100 ml (data from A. Rabinovitch, A. Gutzeit, M. Kikuchi, E. Cerasi, A. E. Renold, to be published)

releases insulin by interacting with a cell-membrane glucoreceptor, or via its intracellular metabolism. This uncertainty will be reflected by the use, in the following, of the term “glucose recognition unit” of the B-cell, glucose standing for glucose as such or any of its metabolites.

It has often been suggested that the initial and late insulin responses to glucose may have different mechanisms, *e.g.* the initial one being controlled by a glucoreceptor, the late one more influenced by B-cell glucose utilization. If one considers the glucose dose-response characteristics of the initial and the late insulin responses, however, it may be seen that the K_m for these two functions is very similar both in man and in a variety of experimental models (Fig. 1), while the capacity (V_{max}) of the late re-

sponse is usually somewhat greater. Thus, whatever cellular mechanisms are involved in these two phases of insulin release, their affinity to glucose seems to be identical. It therefore seems more logical, in the present state of knowledge, to suggest that the action of glucose on the initiation of insulin release is uniform with time, only one recognition unit being involved. The marked, time-bound changes that are known to occur in the final response, *i.e.* in insulin secretion rate, may be due to the modulation of the signal elicited by glucose in the B-cell. This latter phenomenon will be discussed later in this review.

Once the increase in extracellular glucose concentration is recognized by the B-cell, how is this information transmitted further within the cell? Although many other possibilities have been proposed or can be envisaged, I shall limit the discussion to the two which presently seem most evident: cyclic AMP and calcium ions.

It has been known for some years that agents which supposedly increase the B-cell cyclic AMP content also enhance the secretion of insulin [15–17]. Therefore it has been accepted that the adenylate cyclase-cyclic AMP system of the B-cell may be involved in insulin release. Based on indirect evidence, we proposed some years ago that the simplest way of interpreting the data was to assume that glucose acts by increasing the levels of cyclic AMP in the cell [8]. This suggestion has been criticized, mainly

because of the inability of some groups to measure effects of glucose on cyclic AMP content of the islets [18–20]. Since then, both we [21, 22] and others [23] have succeeded in demonstrating that this does indeed occur. Our results will be summarized in the following.

Fig. 2. Time-course of the effect of glucose (5.0 mg/ml) on cyclic AMP accumulation in isolated rat islets (from 22, with the courtesy of the Journal of Biological Chemistry)

Fig. 3. Prolonged time-course of the effect of glucose (5.0 mg/ml) on cyclic AMP accumulation in isolated rat islets with (triangles) and without (filled circles) addition of 0.1 mM 3-isobutyl-1-methylxanthine (IBMX). Open circles denote results obtained in the presence of glucose 0.6 mg/ml with IBMX (from 22, with the courtesy of the Journal of Biological Chemistry)

The ATP pool of isolated rat islets was labelled by preincubating the tissue with ^3H -adenine, and the rate of conversion of ATP to cyclic AMP was measured in a following incubation. This method gives a measure of the net accumulation of cyclic AMP (*i.e.* production rate minus rate of degradation) in the tissue or incubation medium. It could be shown that within seconds to a few minutes after raising the glucose concentration of the incubation medium, an increase occurred in the islet content of cyclic AMP

Fig. 4. Reversibility of the action of glucose on cyclic AMP accumulation in rat islets. Islets were incubated for 3 min with glucose 28 mM and IBMX 0.1 mM. At that time, the volume of the incubate was increased so that the final glucose concentration was decreased to 3.4 mM. IBMX concentration was kept constant throughout (from 22, with the courtesy of the Journal of Biological Chemistry)

(Fig. 2). The action of high glucose on cyclic AMP accumulation was sustained throughout a 2 hr incubation: there were no marked changes similar to those typical for the insulin secretion rate (Fig. 3). Furthermore, the glucose effect on cyclic AMP was rapidly reversible by lowering the glucose concentration, even in the presence of a phosphodiesterase inhibitor (Fig. 4). Finally, the L-isomer of glucose was totally inactive, and the accumulation of cyclic AMP in the rat adipocyte was not influenced by D-glucose [22]. Artefactual effects of glucose on the islet cyclic AMP measurements were excluded by the demonstration that the total, extractable cyclic AMP

content of the tissue (as measured by the protein kinase binding assay) is also augmented by glucose [22] and that the hexose does not alter the specific radioactivity of the islet ATP pool (preliminary observation). Thus, it can be stated with confidence that

glucose rapidly raises the islet content of cyclic AMP, preceding in our incubation conditions the appearance of insulin in the medium.

How does glucose (or its metabolic products) influence the cyclic AMP levels in the islets? Present evidence does not allow the selection of one (or combination) of the following possible mechanisms: direct stimulation of the adenylate cyclase, enlargement of a specific ATP pool serving as substrate for the cyclase, or inhibition of the phosphodiesterases. Although attempts have been made to measure some of these steps, none can be currently excluded [24, 25].

Whatever the mechanism by which glucose may augment the B-cell cyclic AMP levels, are these direct-

Fig. 5. Effect of different concentrations of glucose on accumulation of cyclic AMP in rat islets, efflux of the nucleotide into the medium, and secretion of insulin over a 60 min incubation. Triangles denote incubations in the presence, and circles in the absence of, 0.1 mM IBMX (from 22, with the courtesy of the Journal of Biological Chemistry)

Fig. 6. Effect of theophylline on glucose-induced insulin release from perfused rat islets. Note the immediate enhancing action of theophylline (data from A. Rabinovitch, A. Gutzeit, M. Kikuchi, E. Cerasi, A. E. Renold, to be published)

ly related to the action of the sugar on insulin release? Fig. 5 shows the correlation that exists between the release of insulin and the outflow of labelled cyclic AMP into the medium over a 60 min incubation, in the presence of varying glucose levels. Accumulation of cyclic AMP in the medium rather than in the tissue was selected since only the former is a cumulative parameter, comparable to the accumulation of insulin. The correlation between the two parameters is striking, both the cyclic AMP and insulin responses to glucose showing identical K_m values. This finding suggests, but certainly does not prove, that the recognition unit of glucose for insulin release and that for activation of cyclic AMP accumulation may be identical; in other words, that cyclic AMP may be

mediating the glucose action to the site of insulin release.

A major problem when trying to clarify the role of cyclic AMP in the release of insulin is that, when exogenous cyclic AMP or agents that are supposed

to increase the cellular level of the nucleotide are added to islet preparations, insulin secretion is enhanced only in the presence of high concentrations of glucose, while the effect together with basal concentrations of glucose is negligible [26]. Thus, under these circumstances, the K_m of the insulin response to glucose is not modified while the V_{max} is increased. These findings have usually been interpreted as evidence against the role of cyclic AMP in initiating insulin release, assigning to the nucleotide the role of potentiator of secretion. Already at this stage a distinction has to be made: this potentiation must be different from the glucose induced potentiation that will be discussed later in this paper, since the latter requires a time-lag, while the effect of, e.g., theophylline is immediate (Fig. 6).

Although this view has gained wide acceptance, some recent findings raise serious doubt as to the validity of the argument. Thus, as shown in Fig. 7, low concentrations of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine have only moderate effects on the islet cyclic AMP content by themselves (at low glucose), but still enhance the insulin response to glucose, and this is due to the fact that the glucose-induced increase in cyclic AMP accumulation has been amplified by the drug. From these experiments it is evident that the rate limiting factor in insulin secretion is not glucose as such (compare insulin release at high glucose with and without phosphodiesterase inhibition) but glucose-induced cyclic AMP generation. In another set of experiments, islets were incubated with cholera toxin, a well-known adenylate cyclase stimulator (Fig. 8). With increasing concentrations of the toxin, and in the presence of basal glucose, cyclic AMP generation was augmented, but only a minor increase in insulin release could be detected. When the glucose content of the medium was raised, insulin release was enhanced in the presence of the toxin, and this was accompanied by a marked increase in cyclic AMP accumulation, above the levels induced by the toxin alone. All these findings suggest either that agents other than glucose raise the cyclic AMP level in non-B-cells in the islet, or, which seems more logical to us, that the B-cell contains at least two pools of adenylate cyclase (Fig. 9), of which only one is specific for insulin release and is stimulated by glucose. The enhancement of the glucose effect by methylxanthine derivatives may be caused by amplification of cyclic AMP generation due to glucose; enhancement induced by exogenous cyclic AMP, cholera toxin, glucagon etc. might either represent spill-over of cyclic AMP from the non-specific to the active pool, or competition of both pools of cyclic AMP for common phosphodiesterases (theophylline-

Fig. 7. Effect of 3-isobutyl-1-methylxanthine (IBMX) on insulin release and cyclic AMP efflux from isolated rat islets incubated for 60 min

Fig. 8. Effect of cholera toxin on insulin release and cyclic AMP efflux from isolated rat islets incubated for 60 min (data from V. Grill and E. Cerasi, to be published)

like effect). Whether these hypothetical adenylate cyclase pools are all located at the cell membrane cannot be assessed as yet, but the possibility that the glucose-stimulated compartment may be an intracellular one should be kept in mind (endoplasmic reticulum? granule membrane?). Recently, data have

Fig. 9. Hypothetical model of cyclic AMP compartments in the B-cell. Arrows mean stimulation, —| denotes inhibition. See also text

Fig. 10. The interplay between cyclic AMP and calcium in the B-cell. Two possibilities are visualized: A) Sequential action. Glucose-induced cyclic AMP accumulation mobilizes calcium from intracellular stores (mitochondria etc.) which then activates insulin release. B) Parallel action. Cyclic AMP acts directly on insulin release, an action which is enhanced by the increase in cytosolic calcium level. Glucose also diminishes the efflux of calcium from the cell

been presented suggesting the presence of distinct compartments of cyclic AMP in the toad bladder [27], which may offer some support to our hypothesis.

Another factor that plays a key-role in secretory processes is calcium ions. The evidence for the involvement of calcium in insulin secretion has recently

been reviewed [28]. It has been shown that increasing the glucose concentration of the incubation medium reduces rapidly the efflux of radioactive calcium from islets previously loaded with $^{45}\text{Ca}^{++}$, and this has been accepted as evidence that glucose increases the cytosolic calcium ion level. The question can be raised, however, whether decreasing the outward transport through the cell membrane of calcium ions alone can augment their concentration in the cytosol. Indeed, it is known both from other tissues and the pancreatic islets, that several subcellular organelles and especially the mitochondria, have a high affinity and capacity for calcium [29, 30]. Thus, release of calcium from intracellular stores probably has more profound effects on its cytosolic level, and it has been claimed that cyclic AMP is one of the factors that liberate the ion from such stores [31]. Malaisse has produced evidence indicating that cyclic AMP may have a similar effect on the pancreatic islet, and has postulated that the amplifying action of *e.g.* theophylline on insulin release may be due to its mobilization of calcium from intracellular stores [26]. If one takes into account our demonstration of the effect of glucose on cyclic AMP levels in the islet, the following hypothesis may be proposed for the glucose-induced insulin release (Fig. 10): glucose rapidly augments the level of cyclic AMP, which *i.a.* releases calcium ions from sites where it is bound, thus increasing its cytosolic level; another (independent?) effect of glucose limits the loss of cytosolic calcium through the cell membrane; insulin release is controlled either by calcium alone, the sequence of events then being glucose $\rightarrow$ cyclic AMP $\rightarrow$ calcium, or synergistically by cyclic AMP and calcium (permissive effects?), both agents acting in a parallel manner on secretion. Although recent findings demonstrating considerable insulin release by a calcium ionophore in the absence of glucose may support the first alternative [32, 33], it has also been claimed that the ionophore increases the islet cyclic AMP content in glucose-free medium [32], the choice between the above two possibilities thus becoming impossible at present.

Potential of Insulin Release by Glucose

It has been postulated in the above discussion that the effect of glucose on the B-cell as an initiator of insulin release is uniform in time. However, it is well established that the insulin response to constant stimulation by glucose is biphasic, marked changes in secretion rate occurring over short periods of time. We have recently proposed [34], in contrast to suggestions from other authors and previously from our-

selves [35, 36] that this feature of insulin release may be due to the superposition of stimulatory and inhibitory events with different time-kinetics. Thus, it has been shown both *in vitro* [37] and in man [38]

Fig. 11. Enhancement of insulin response by pretreatment with glucose in man. The horizontal bars on the abscissa symbolize two glucose infusions (500 mg/kg as priming and 20 mg/kg/min during 60 min) performed with a 40 min interval

Fig. 12. Modification of the glucose-insulin dose-response relationship by pretreatment with glucose in man. The insulin response to glucose infusions with varying doses was measured with and without a prior infusion of 500 mg/kg + 20 mg/kg/min of glucose for 60 min (see Fig. 11). Early response refers to values obtained at 10 min, late response at 60 min

that repetition of glucose stimulations, with short time intervals, results in diminution of the second response, the degree of inhibition being directly related to the magnitude of the first response [38]. The mechanism of this inhibition is not known, but might be mediated by the released insulin itself [39]. It is possible that the decrease in insulin release that follows the initial peak during constant glucose stimulation is due to this phenomenon of inhibition.

More important than this short-lasting inhibition is the enhancement of insulin release that appears when the islets have been exposed for some time to hyperglycemia. Fig. 11 shows a typical experiment in man, where it is seen that both the initial and late phases of the insulin response to glucose are enhanced by the previous administration of glucose. This synergistic effect of glucose on its own action was shown to be time dependent both *in vitro* [2] and in man [34], its time-lag in onset and its duration lasting considerably more than those of the phase of inhibition [34]. The kinetic aspects of this state of potentiation induced by glucose were studied in some detail [40]. It could be shown that pretreatment with glucose probably does not change the affinity of the second insulin response for glucose, but that it increases its capacity (Fig. 12). In these terms, the glucose pretreatment causes true potentiation of the second response, and is analogous to the effect of arginine [41] or theophylline [26], but for the time aspects: the latter act instantaneously, in contrast with the time-lag needed for the effect of glucose. Insulin release during pretreatment with glucose is obviously not involved in the generation of this state of potentiation, since administration of adrenaline, which blocked the insulin response to glucose, did not inhibit the later appearance of enhancement [42]. We have therefore tentatively suggested that glucose-induced potentiation of insulin release may be generated by some process linked to glucose utilization in the B-cell, by this implying that it is distinct from the "glucose recognition unit". Another finding that supports this view is that the sensitivity of the potentiation system to glucose is very different from that of the initiation of insulin release (see also below). Thus, while release of insulin has its threshold for glucose at concentrations around 100–110 mg/100 ml, potentiation could not be generated unless the pretreatment hyperglycemia reached the levels of 250–300 mg/100 ml [40].

What mechanism mediates the glucose-induced potentiation in the B-cell? Presently no biochemical data are available that permit the formulation of a hypothesis. If our data on the effect of incubation time on cyclic AMP accumulation in the islets (Fig. 3) can be extended to the above studies on potentiation, it

Fig. 13. Enhancement of glucagon-induced insulin release by pretreatment with glucose in man. The i.v. injection of 1 mg of glucagon (arrow) was preceded by a glucose infusion terminated 50 min earlier. Panel B shows the control experiment

Fig. 14. Hypothetical model for the B-cell recognition of glucose as an initiator and as a potentiator of insulin release. See also text

becomes evident that enhancement is not due to major augmentation with time of the effect of glucose on the cellular level of the nucleotide. Alternatively, the effect of cyclic AMP on insulin release may be ampli-

Fig. 15. Plasma insulin response to glucose in early stages of diabetes. *Left panel:* Glucose infusion in normal and mildly diabetic man. 500 mg/kg glucose was given i.v. at 0 min, followed by the infusion of 20 mg/kg/min (in normal man) or 10 mg/kg/min (in the diabetic) for 60 min. *Right panel:* Injection of 1.5 g/kg of glucose at 0 min into a femoral vein of anesthetized mice and *acomys*. Blood was sampled from the retroorbital plexus (data from A. Gutzeit, A. Rabinovitch, P. Studer, P. Trueheart, E. Cerasi, A. E. Renold, to be published)

Fig. 16. Dose-response relationship of glucose-induced late insulin response in the various developmental stages of human diabetes (left; data from 42), and in islets of *acomys cahirinus* incubated during 120 min (right; data from A. Gutzeit, A. Rabinovitch, C. Karakash, W. Stauffacher, A. E. Renold, E. Cerasi, to be published)

fied. Indirect evidence that this may be the case is shown by the fact that the action of glucagon (which acts by generating cyclic AMP) on insulin release is also enhanced by pretreatment with glucose (Fig. 13)

Fig. 17. Effect of different glucose concentrations on insulin release (left panel) and glucose utilization in 60 min incubates of isolated rat (open circles) and *acomys* (filled circles) islets. Glucose utilization was estimated by measuring the rate of incorporation of ^3H from ^3H -5-glucose to water (data from G. Cuendet, A. Rabinovitch, E. Cerasi, A. E. Renold, to be published)

[42]. This latter alternative has been depicted in the hypothetical model of Fig. 14, but evidently experimental confirmation has to be awaited. (Readers interested in the quantitative and kinetic aspects of the interplay between initiation, inhibition and potentiation of insulin release on stimulation by glucose are referred to our paper, ref. 34).

Insulin Release in the Diabetic Syndrome

As stated in the introductory lines, the insulin response to glucose is diminished or obliterated in the various stages of the diabetic syndrome. It has been said often (also by ourselves) that the defective release of insulin concerns mainly the initial phase of the response. However, it can be shown in many patients with mild, maturity-onset diabetes, and in experimental models such as the spiny mouse (*Acomys cahirinus*), in which glucose tolerance is diminished, that the kinetics of insulin release are not necessarily grossly modified, but that the totality of the response is reduced when certain types of glycemic stimulation are applied (Fig. 15). Therefore, and in accordance with our statement above on the uniformity with time of the initiating action of glucose on insulin release, we suggest that the recognition of glucose at any time

Fig. 18. Insulin release and efflux of cyclic AMP from perfused islets of rat and *acomys*. The islets were preincubated for 60 min with ^3H -2-adenine and then placed in perfusion chambers. Two min pooled aliquots of the perfusate were purified as in ref. 22, and the radioactivity of cyclic AMP measured (data from A. Rabinovitch, V. Grill, A. E. Renold, E. Cerasi, to be published)

during the stimulation is impaired in the diabetic. We have also shown that this impairment probably corresponds to a decrease in the sensitivity of the islet for glucose since, both in normal subjects with low insulin response and in some maturity-onset diabetics [43, 44], and also in the *Acomys* [45], the maximal response of the pancreas to glucose may be normal, but the threshold glucose concentrations are higher than in controls (Fig. 16). Although it may be hazardous to draw firm conclusions from studies in man, in

conditions where the islets respond to glucose with decreased insulin output, a normal rate of glucose utilization does occur (Fig. 17). In the preceding sections of this review, we implied that cyclic AMP accumulation mediated the recognition of glucose as an initiator of insulin release. The data shown in Fig. 18 suggest that, in the *Acomys*, the defect in the transmission of the hyperglycemic stimulus is prior to the formation of cyclic AMP, either at the recognition site of glucose (or its metabolites) or at the link(s)

Fig. 19. a) Potentiation of insulin release by pretreatment with glucose in low insulin responders. The right panel shows the control experiment. Two consecutive glucose infusions were performed: 250 mg/kg as priming followed by 10 mg/kg/min from 0 to 60 min, and 500 mg/kg + 20 mg/kg/min from 100 to 160 min (same dose as for the control infusion). b) Potentiation of insulin release in mildly diabetic subjects. Two identical glucose infusions (500 mg/kg as priming, 10 mg/kg/min as constant infusion) were performed at 0–60 and 130–190 min

whom *e.g.* a maximal insulin response practically never can be achieved, we believe that the basic defect in the mild forms or initial stages of diabetes indeed manifests itself as decreased sensitivity of the recognition unit for glucose (increase in K_m). There is no evidence in the literature indicating that the glucose utilization of islets from animals deficient in their insulin response should be grossly modified. Recent experiments with islets of *Acomys* confirm that, under

between this site and the adenylate cyclase system. Whether this finding applies also to the islet of the human diabetic is obviously unknown, although earlier findings with the use of theophylline may suggest the existence of an analogous situation in man [46].

An obvious question that arises is whether all of the complex effects of glucose on insulin release that were discussed above are modified in diabetes. Several findings suggest that potentiation of the insulin re-

sponse by glucose may not necessarily be impaired in the diabetic syndrome: firstly, our demonstration that the potentiating effect of glucose may be dissociated from its insulin releasing effect [42]; secondly, the fact that in many mildly diabetic subjects, with *time* after glucose administration, major insulin secretion may occur (see Fig. 15); thirdly that the glucose utilization of an islet deficient in insulin response (*Acomys*) may be normal (Fig. 17). Indeed, it could

glucose (Fig. 20, compare with Fig. 16). These findings support the suggestion made earlier that the sites in the B-cell responsible for the recognition of glucose as an initiator of insulin release (over cyclic AMP), and those for the recognition of its time-bound potentiating action are distinct ones, and that only the former are defective in the initial stages of diabetes mellitus (Fig. 14).

In conclusion, the initial defect in the diabetic B-cell seems to consist in a limited modification (mutation?) of a specific step in the recognition of hyperglycemia as the acute trigger of the processes that lead to release of insulin. In spite of its limited character (decrease of sensitivity), its strategic location may cause important delays in the feed-back loops that regulate the glucose homeostasis of the organism, and therefore be sufficient to impair the glucose tolerance. The recent findings presented here justify our previous suggestion of regarding diabetes mellitus as a disorder of cellular information transmission [8].

Acknowledgements. The Minkowski Award is usually given to researchers between two ages: they have barely emerged from the period of apprenticeship, and only recently started their role of scientific guide. I am no more original than my predecessors in this respect, and my feelings of gratitude therefore are directed vertically in both directions. Those who have followed our work over the past decade will recognize that most of the ideas presented in this review were developed in collaboration with Dr. R. Luft. I owe him a large amount of gratitude for having initiated me to endocrinology and research, for his intense tutorship during our early work, and for the most stimulating collaboration and companionship he has offered me throughout many years. Dr. S. Efendic deserves my thanks not only for our collaboration, but also for having brought some of the sunshine of my geographic origins to the 60th parallel. My younger colleagues, Drs. V. Grill, U. Adamson and A. Widström, have stimulated and produced ideas and data, cited or not cited in this review, that make the life of a researcher most agreeable. My sincere thanks and appreciation to them. Some of the most recent findings presented here have been produced at Dr. A. E. Renold's laboratory in Geneva. My warmest thanks are due to my new collaborators, Drs. A. Rabinovitch, A. Gutzeit, M. Kikuchi and G. Cuendet. The majority of the studies reviewed have been supported by grants from the Swedish Medical Research Council, the Knut and Alice Wallenberg Foundation, and the Riksbankens Jubileumsfond. The author was recipient of a Pfizer-Europe Travel Fellowship (1973).

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POTENTIATION OF GLUCOSE - INDUCED INSULIN RELEASE BY PREINFUSION OF GLUCOSE

Fig. 20. Dose-dependency of the potentiating action of glucose on glucose-induced insulin release. The insulin response to a fixed-dose glucose infusion was measured with and without prior glucose infusions at varying doses. Total insulinogenic index was calculated as the total plasma insulin area of the glucose infusion divided by the blood glucose area. The index of the control experiment is taken as 100%. The abscissa shows the mean blood glucose level achieved during the 100 min that preceded the second glucose infusion test

be shown that pretreatment with glucose enhanced the insulin response to a second glucose challenge in subjects with low insulin response and in mildly diabetic patients [47] (Fig. 19). The type of amplification was similar to that seen in normal subjects, *i.e.* increase in V_{max} of the response. More important, the sensitivity of the subjects to the potentiating action of glucose was either increased (low insulin responders) or normal (mild diabetics), contrasting with the markedly diminished sensitivity to the initiating action of

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