
Section 2: Biphasic Insulin Release: Pools and Signal Modulation

Modeling Phasic Insulin Release

Immediate and Time-Dependent Effects of Glucose

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The cellular and molecular mechanisms of insulin secretion are being intensively investigated, yet most researchers are seemingly unaware of the complexity of the dynamic regulation of the secretion. In this article, we summarize studies of the physiology of insulin secretion performed over several decades. The insulin response of perfused islets of rats, perfused rat pancreas, or that of a human, to a square-wave glucose stimulus is biphasic, a transient first-phase response of 4- to 10-min duration followed by a gradual rise in secretion rates (second-phase response). Several hypotheses have been proposed to account for the phasic nature of insulin secretion; they are briefly discussed in this review. We have favored the hypothesis that nutrient stimulators such as glucose, in addition to a primary and almost immediate secretory signal, with time induce both stimulatory and inhibitory messages in the β -cell, and those messages modulate the primary insulinogenic signal. Indeed, studies in the rat pancreas and in humans have demonstrated that short stimulations with glucose generate a state of refractoriness of the insulin secretion, which we have termed time-dependent inhibition (TDI). Nonnutrient secretagogues such as arginine induce strong TDI independent of the duration of stimulation. Once the agent is removed, TDI persists for a considerable period. In contrast, prolonged stimulations with glucose (and other nutrients) lead to the amplification of the insulin response to subsequent stimuli; this can be demonstrated in the perfused rat pancreas, in perfused islets from several rodents, and in humans. We have termed this stimulatory signal time-dependent potentiation (TDP). The generation of TDP requires higher glucose concentrations and prolonged stimulation; the effect is retained for some time after cessation of the stimulus. Of major interest is the observation that, while the acute insulin response to glucose is severely reduced in glucose-intolerant animals and humans, TDP seems to be intact. The cellular mechanisms of TDI and TDP are poorly understood, but data reviewed here suggest that they are distinct from those that lead to the acute insulin response to stimuli. A

model is proposed whereby the magnitude and kinetics of the insulin response to a given stimulus reflect the balance between TDP and TDI. Researchers studying the cellular and molecular mechanisms of insulin release are urged to take into consideration these complex and opposing factors which regulate insulin secretion. *Diabetes* 51 (Suppl. 1):S53-S59, 2002

The strong emphasis of the past several years on the molecular mechanisms that govern cell function, albeit fully justified, has nevertheless detracted attention away from the integrated physiological regulatory processes that are responsible for the macrocosm in which organs and whole organisms function. The regulation of insulin secretion is a good example. Major progress has been made regarding the molecular mechanisms of secretory events, both in general terms and as applied to the pancreatic β -cell. Yet the study of the in vivo physiology of insulin release has permitted unraveling an unsuspected degree of complexity, not always apparent when investigating single β -cells (not to mention subcellular components). Here we summarize some of the information gained over several decades of studying the physiology of insulin release, which led to the formulation of interesting concepts on the dynamic regulation of β -cell function.

Although many nutrients, hormones, and neural stimuli modulate the secretion of insulin, glucose must be regarded as the main regulator of insulin synthesis and release. The hallmarks of the β -cell response to an increase in extracellular glucose concentration are (a) the rapidity of insulin release, (b) the high degree of sensitivity to the stimulus, (c) the large amplitude range of the responses, and (d) the oscillatory nature of the secretion. However, all these features are highly dependent on the experimental system used. Thus, whereas as low a glucose stimulus as 6.7 mmol/l is fully sufficient to elicit a rapid and distinct insulin response in the perfused rat pancreas (Fig. 1), in isolated islets, higher concentrations and longer exposure times are necessary. In humans, in the few studies where blood could be sampled from the portal vein (1-3), the time lag from the rise of the glucose concentration to the peak insulin response was as short as 60 to 120 s; even in a peripheral vein, the peak response is

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IGT, impaired glucose tolerance; TDI, time-dependent inhibition; TDP, time-dependent potentiation.

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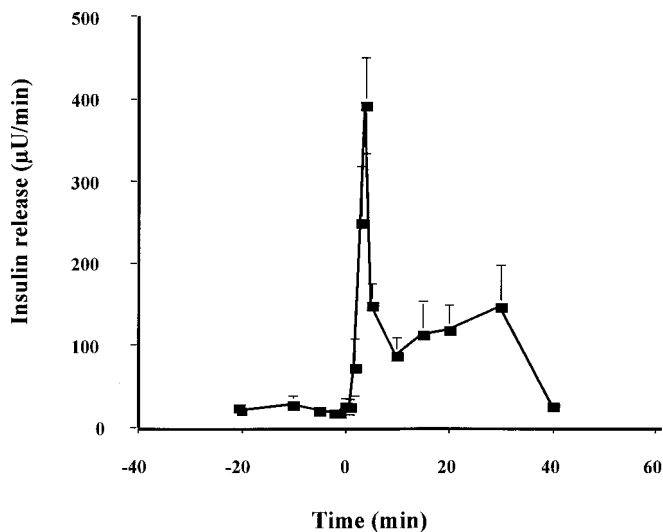


FIG. 1. Biphasic insulin response to glucose in the isolated, perfused rat pancreas. The pancreas was perfused with 3.3 mmol/l glucose throughout, except during the 0- to 30-min period, when it was raised to 6.9 mmol/l.

observed 3 to 5 min after initiating a glucose infusion (Fig. 2). Likewise, the range of response amplitudes is wider the more physiological the study system. For example, it is almost impossible to achieve maximal insulin responses over 60 min by intravenous glucose infusions in humans even with blood glucose concentrations in excess of 40 mmol/l, whereas saturation is reached with 15 to 20 mmol/l glucose in isolated islets, and the amplitude of the response (fold increase over basal secretion) is markedly lower (4). This reduction in the plasticity of β -cell responsiveness becomes even more striking when dispersed β -cells or cell lines are studied.

Dose-response characteristics of glucose-induced insulin release: pools or signal modulation? In broad terms, two different explanations have been proposed for the dose-response characteristics of insulin secretion. The first suggests that the magnitude of the intracellular signals that induce insulin release depend on glucose dose, each β -cell responding in a graded manner to increasing glucose concentrations. Support for this comes from the

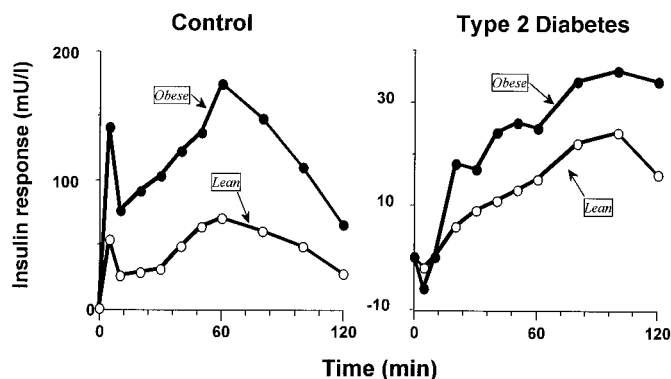


FIG. 2. Plasma insulin response to glucose infusion in humans. Glucose was given at the dose of 500 mg as an intravenous injection at time 0, followed by the infusion of 20 mg/min during the next 60 min. Note that the x-axis in the right panel (type 2 diabetic subjects with fasting plasma glucose 7–10 mmol/l) is fourfold expanded compared to the left panel (control subjects). \circ , results in lean subjects; \bullet , results in moderately obese subjects. Adapted from Cerasi (4).

observations that the electrical activity and Ca^{2+} fluxes of a β -cell show glucose dose dependence, and that insulin release from single β -cells measured by various techniques (see article by Rorsman et al. in this issue) is similarly modulated by varying glucose concentrations. The ionic coupling that exists between β -cells, to the extent that an islet may function as a single electrophysiological unit driven by a “pacemaker” (5), strengthens this view.

The alternative view is that each β -cell functions in an “all-or-none” mode and responds to a given glucose concentration by discharging all its releasable pool of insulin. Because each β -cell has a different sensitivity (threshold) for glucose, the higher the glucose concentration, the greater the number of β -cells that are recruited to secrete, hence the sigmoid shape of the glucose-insulin dose-response curve. Pipeleers and colleagues (6,7) have been the major proponents of this view; they have indeed demonstrated that isolated dispersed β -cells secrete insulin at varying threshold glucose concentrations. A variant of this view is that the β -cell may contain several pools of β -granules, discharged at varying glucose concentrations (see below; also, article by Bratanova-Tochkova et al. in this supplement).

To the best of our knowledge, no data exist on the in situ responsiveness of β -cells within the intact pancreas. Thus, it is not clear to what extent the in vitro demonstration of functional heterogeneity reflects a physiological β -cell reality, rather than an artifactual modification of β -cell function, especially when cells are dispersed. Even if true differences in glucose sensitivity may exist among β -cells, it is likely that the cell with the highest sensitivity to glucose entrains all the other β -cells to which it is electrically coupled, as suggested in a recent study by Jonkers and Henquin (8). Clearly, more sophisticated in situ methodology is needed to distinguish between these options.

Biphasic insulin release. The most used method to investigate the kinetic characteristics of glucose-induced insulin secretion has been the so-called square-wave stimulation, in which the pancreas is exposed to a rapid rise in glucose concentration which is then kept constant for the desired duration; its clinical counterpart is the hyperglycemic clamp with primed-continuous infusion of glucose. Admittedly, square-wave stimulation is not physiological, since such abrupt and marked increases in blood glucose do not occur in nature. Indeed, when food or even concentrated glucose solutions are ingested, blood glucose and plasma insulin rise gradually, and no clear phasicity of the insulin response can be detected. Nevertheless, the fact that the different phases of insulin secretion may have different regulators, that there may be metabolic impacts to phasic release, and that, in the early stages of type 2 diabetes, first-phase insulin response is preferentially damaged (see several articles in this supplement), indicate that biphasic insulin secretion is a real characteristic of β -cell function.

The insulin secretory response of type 2 diabetic patients with even modest hyperglycemia is characteristically decreased and delayed (Fig. 2). This is observed both in overtly diabetic subjects and in persons with glucose intolerance only; however, the higher the fasting blood glucose, the flatter the insulin response to glucose, and, in many patients, glucose initially even induces reduction in

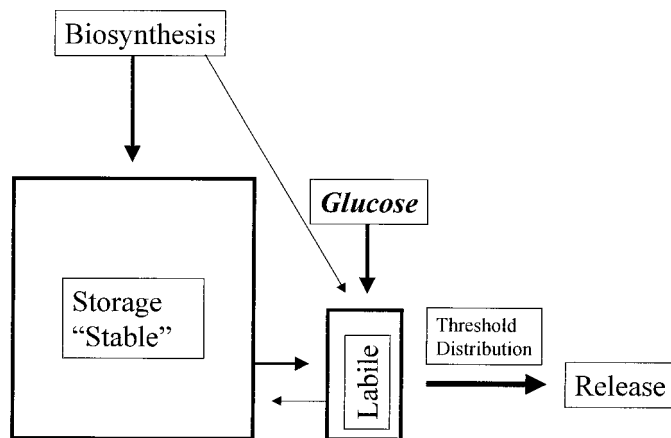


FIG. 3. Two-compartment model of insulin secretion. See text for explanation. Adapted from Grodsky (15).

plasma insulin levels (for review, see (4)). First-phase insulin release has a lower glucose threshold than late-phase response (9). In type 2 diabetes, first-phase insulin release shows decreased maximal capacity (V_{max}), whereas second-phase response has a rightward shift (increased apparent K_m) (10). Nevertheless, because saturation of the insulin response to glucose is hardly ever reached in humans, conclusions regarding the kinetic nature of the secretory defect in diabetes (K_m versus V_{max} changes) need caution. In islets isolated from *Acomys cahirinus*, a rodent with glucose intolerance, glucose dose-response studies demonstrated that, compared with rat islets, the V_{max} of the first-phase response was reduced, whereas second-phase response showed increased K_m (11), findings supporting those in human type 2 diabetes.

Biphasic dynamics of glucose-induced insulin release: pools or signal modulation? The existence of biphasic insulin release in response to glucose was first reported by Grodsky and colleagues in vitro and by our group in vivo in the 1960s (12,13). Despite the intervening four decades, the mechanisms underlying the biphasic response of the β -cell remain poorly understood. In analogy with the discussion above on the dose-response relationship of glucose-induced insulin secretion, two main lines of explanation for biphasic release have been put forth over the years. The first, developed in a series of publications by Grodsky's laboratory (14–16), and modeled by us to simulate insulin responses to glucose infusion in humans (17), assumes that the β -cell contains two distinct pools of insulin granules: a small, labile pool accessible for immediate release and a larger pool that feeds slowly into the labile pool (Fig. 3). According to this model, the transient nature of first-phase insulin release is the consequence of the exhaustion of the labile pool early during glucose stimulation; with enough time, the compartment is refilled by transfer of granules from the large stored insulin pool, which then allows for second-phase insulin secretion. A modern version of this model has been presented by Daniel et al. (18; see also article by Bratanova-Tochkova et al. in this supplement), who succeeded in defining some of the molecular characteristics of readily releasable insulin granules.

To explain the time-kinetics of first-phase insulin release, the labile pool must be small and finite, its exhaus-

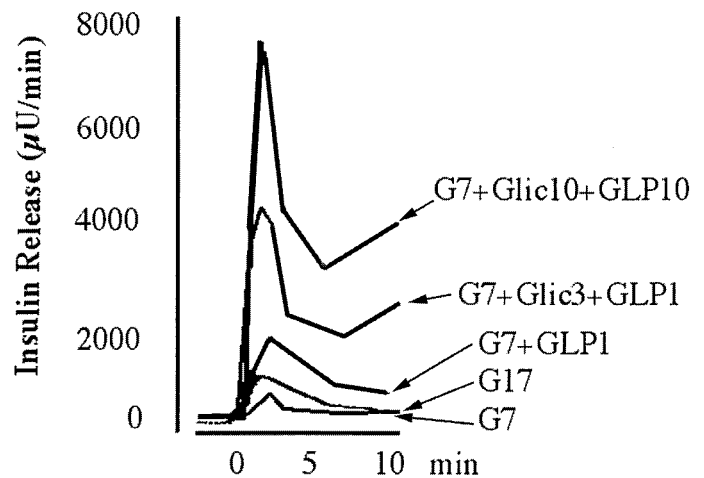


FIG. 4. First-phase insulin release in the perfused rat pancreas during different experiments with increasing strength of stimulation. G7, 7 mmol/l glucose; G17, 17 mmol/l glucose; G7+GLP1, perfusion with 7 mmol/l glucose and 1 nmol/l GLP-1; G7+Glic3+GLP1, perfusion with 7 mmol/l glucose, 3 μ mol/l sulfonylurea (gliclazide), and 1 nmol/l GLP-1; G7+Glic10+GLP10, perfusion with 7 mmol/l glucose, 10 μ mol/l gliclazide, and 10 nmol/l GLP-1. Note the greater than 10-fold change in the amplitude of first-phase response despite similar kinetic characteristics. Composite figure from several unpublished experiments.

tion corresponding to the nadir between the two phases of insulin secretion (Fig. 1). A problem emerges when the effect of varying concentrations of glucose and other stimuli on first-phase release is examined: as shown in Fig. 4, at varying glucose levels and with the addition of amplifiers (e.g., glucagon-like peptide-1 [GLP-1] or sulfonylureas), the shape and the transient nature of the first peak remain unchanged, while peak secretion rates increase dramatically with escalating stimulus intensity. Thus, the apparent size of the putative labile pool is not constant, since it allows for a wide range of transient insulin responses whose magnitude is determined, without time lag, by the intensity of the stimulus. To accommodate at least partly for such observations, it was suggested that different insulin pools may be recruited for secretion at different levels of stimulation (called threshold distribution hypothesis [14,15,19]); alternatively, insulin granules of multiple storage pools may be feeding very rapidly into the releasable pool under given stimulatory conditions (G.W. Sharp, personal communication). While all these suggestions may be reasonable, they require postulating the existence of multiple insulin pools whose behavior can hardly be predicted. To our mind, therefore, pool-related models cease to serve their original purpose, i.e., to provide a simple explanation for the biphasic nature of insulin secretion. We have therefore abandoned the pool model (17,20) in favor of the idea that the changes in insulin secretion rate reflect kinetic modulations of the insulinotropic signal generated by glucose and other secretagogues (so-called signal-modulation hypothesis) (21,22).

Time-dependent effects on insulin secretion: β -cell memories. Grodsky and collaborators were the first to describe in the perfused rat pancreas (12,16), followed by our studies in humans (23–26), that if the pancreas is challenged repeatedly, the insulin response to subsequent stimulations is markedly modified. When a nutrient stimulator of the β -cell such as glucose is used, the type and magnitude of the modulation of subsequent insulin re-

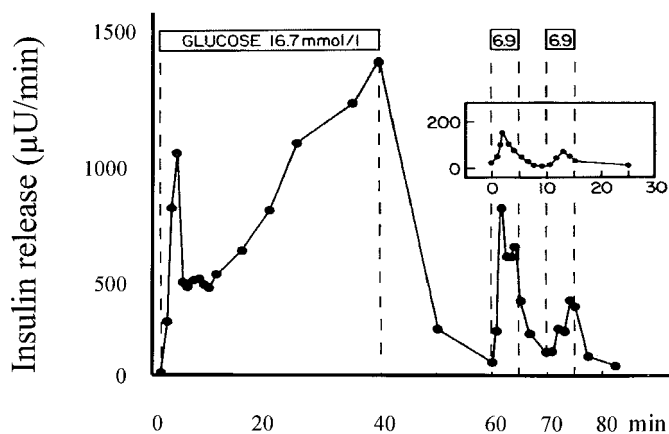


FIG. 5. Coexistence of TDP and TDI in the perfused rat pancreas. Inset: control experiment showing the magnitude of the insulin responses to two consecutive 5-min 6.9-mmol/l-glucose pulses, demonstrating clear TDI. When repeated following a 40-min 16.7-mmol/l glucose priming period, while the 6.9-mmol/l glucose pulses still demonstrated TDI, the amplitude of the insulin responses to these pulses were symmetrically amplified approximately fourfold by the TDP generated during the priming period. Adapted from Nesher and Cerasi (9).

sponses depend on the concentration of glucose and the duration of the stimulation applied: high concentrations and long durations generate amplification of the subsequent insulin responses, while lower concentrations and shorter pulses of stimulation tend to induce a refractory state. Nonnutrient stimuli such as arginine induce exclusively the refractory state, whatever the concentration and duration of the stimulus. We coined the terms time-dependent potentiation (TDP) and time-dependent inhibition (TDI) to describe these events.

Time-dependent inhibition. The interphasic nadir in the rate of glucose-induced insulin release is a unique feature with a reproducible time sequence in square-wave stimulations (27–29). This refractory phase is not observed during an oral glucose load, where islets are subjected to a gradually rising ramp-type stimulus. Nonmetabolizable secretagogues (such as arginine, glucagon, and tolbutamide) administered at a substimulatory glucose level elicit only monophasic insulin responses (28), late-phase response becoming evident only if the glucose concentration is increased (30,31). We suggested that the monophasic dynamics of the insulin response to nonnutrient stimuli are due to the immediate activation of intracellular signals involved in early insulin response, together with generation of TDI signals (9,22). Indeed, with such agents, TDI can be evidenced regardless of the stimulation length (28). If nutrient stimuli are used, to avoid interference by other

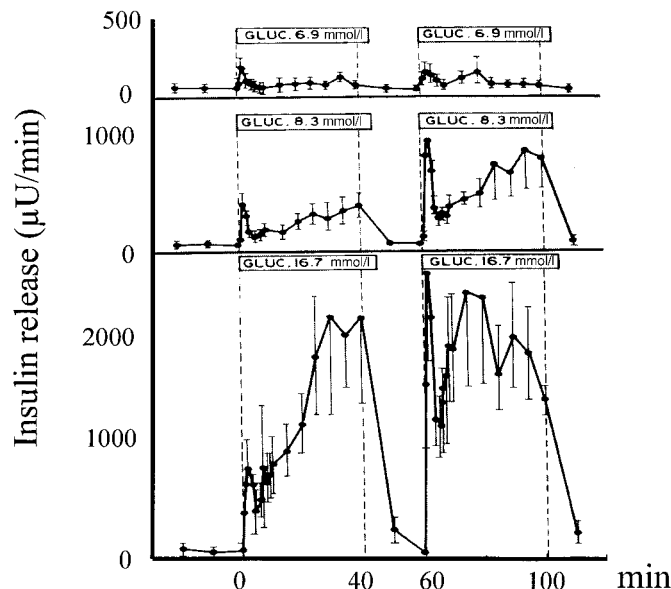


FIG. 6. Glucose dose dependence of TDP. The rat pancreas was stimulated sequentially by two glucose pulses of 40 min. TDP was observed only at 8.3 and 16.7 mmol/l glucose. Adapted from Nesher and Cerasi (9).

synergistic and time-dependent potentiating signals (see below), TDI is best estimated using short pulses of stimuli (9,32). Thus, 10-min pulses were used with 8.3 mmol/l glucose as the primary stimulus, but shorter pulses (5 min or less) were required to demonstrate the expression of the TDI signal at 16.7 mmol/l glucose (28). At lower doses of glucose, TDI could be induced in the isolated rat pancreas at concentrations barely sufficient to elicit an insulin response (Fig. 5). It is of interest that the TDI generated by arginine persisted over prolonged periods (more than 80 min) after withdrawal of the agent (33). Thus, the intracellular mediator(s) responsible for the expression of the TDI signal are not readily eliminated in the β -cell. Unfortunately, the nature of the β -cell mechanisms that generate TDI is not clear; we only know that inhibition of insulin secretion during the TDI-generating glucose pulse did not prevent its induction (32), while elevated glucose concentrations blocked its expression (33). Table 1 summarizes some of the features of TDI.

Time-dependent potentiation. Glucose generates a state of potentiation (TDP) in the β -cell, which expresses itself by the amplification of the insulin response to subsequent stimulations (9). This action of glucose can be demonstrated by applying sequential glucose stimulations,

TABLE 1
Insulin release: characteristics of initiation, TDI, and TDP functions

Feature	Initiation of release	TDI	TDP
Glucose threshold	Low	Low	High
Onset time	Very short	Short	Long
Duration ($t_{1/2}$)	Short	Very long (>80 min)	Long (20–80 min)
Induced by nutrient secretagogues	Yes	Yes	Yes
Induced by nonnutrient secretagogues	Yes	Yes	No
Experimental system	Isolated islets, perfused pancreas, humans	Perfused pancreas, humans	Isolated islets, perfused pancreas, humans
In IGT	Low	Not tested	Present
In type 2 diabetes	Very low/absent	Not tested	Present in early diabetes

where the second insulin response becomes usually two- to fourfold greater than the first one. The signal for TDP is generated by prolonged stimulation with a nutrient secretagogue and is dependent on both exposure time and stimulus dose (9,25,28) (Figs. 5 and 6). TDP displays a finite half-life, the magnitude of the effect declining as the rest period between stimuli extends. In humans, glucose-induced TDP had an apparent $t_{1/2}$ in the order of 60–80 min (25), while in isolated islets of *Acomys cahirinus*, the $t_{1/2}$ was ~20 min (34). Of particular interest is the finding that TDP remained intact in subjects with impaired glucose tolerance (IGT) in whom the acute insulin response to glucose was markedly reduced; in fact, TDP was even more efficient in IGT subjects than in control subjects (24). Also, in the IGT model *Acomys cahirinus*, 30-min priming of isolated islets by 16.7 mmol/l glucose led to ninefold potentiation of the early insulin response to a subsequent glucose stimulus and threefold potentiation of the late response; thus, a prominent first-phase release became evident, thereby normalizing the insulin release dynamics (34). These observations suggest that early-phase insulin release is considerably more sensitive to modulations than the second phase of secretion. A more important conclusion is that the time-dependent regulatory system remains intact in the early stages of type 2 diabetes. Table 1 summarizes some of the features of TDP.

The molecular mechanisms of TDP remain unknown. We found that insulin secretion during the TDP-generating priming period was not a requisite for TDP effect on subsequent stimuli, since omission of Ca^{2+} ions from the glucose priming period, or addition of epinephrine, somatostatin, or diazoxide, all conditions that markedly inhibited the insulin response to the priming pulse, had no effect on the TDP-amplified insulin response of the second stimulation period (23,35,36). As further support for this line of thought, redistribution of insulin granules to locations adjacent to the β -cell plasma membrane could not be observed during TDP generation (37). Thus, the glucose stimulus-secretion signals that control the acute effect of the hexose on insulin secretion and those that mediate its time-dependent actions must clearly be distinct. The presence of normal TDP in IGT is in keeping with this suggestion. (For discussion on the role of protein kinase C, see Neshet et al. in this supplement; several other articles in this supplement also address this and related issues).

Biphasic insulin release: balance between inhibitory and potentiating effects of glucose. We postulated above that biphasic insulin response to nutrient stimuli such as glucose is the result of an interplay between the different priming effects of insulin secretagogues (TDI and TDP). However, since TDI and TDP were defined on the basis of discrete repetitive stimulations interrupted by rest periods, this assumption requires the demonstration that TDI and TDP can coexist and exert their actions simultaneously in the β -cell. Regarding TDI, it was observed that during concomitant stimulation with arginine and glucose, the insulin secretion rate is markedly inhibited despite ongoing glucose stimulation when arginine is removed from the mixture, indicating that arginine-induced TDI was present all along but masked by the strong synergistic interaction between glucose and arginine (9). Similar observations were made in experiments with glucose

alone: when a square-wave stimulus with 16.7 mmol/l glucose was interrupted and transiently replaced by 8.3 mmol/l glucose, again strong inhibition of insulin secretion was observed, the secretion rate falling far below that normally induced by 8.3 mmol/l glucose (R.N. and E.C., unpublished observations). A reverse experimental design was used to demonstrate the presence of TDP while TDI exerts its action (28): a pair of 5-min 6.9-mmol/l glucose pulses displayed the distinctive inhibition of the second insulin response by TDI; when these were preceded by a 40-min 16.7-mmol/l glucose stimulation and a 20-min 3.3-mmol/l glucose rest period, insulin secretion to 6.7 mmol/l glucose was amplified three- to fourfold by TDP action; however, the second pulse was still strongly inhibited by the TDI generated through the first 5-min pulse (Fig. 5). Thus, TDP and TDI signals can exist simultaneously in the β -cell. On the basis of the above evidence, we arrived at the conclusion that the insulin secretion rate at a given time is the composite effect of the dynamic interactions generated by the immediate and time-dependent signals elicited by a secretagogue, the nature, timing, and concentration of the secretagogue determining the final shape and magnitude of the insulin response. We ascribed a more important physiological role to TDP since—according to the above assumptions—it would largely control the longer-lasting late phase of insulin secretion. If this is correct, there should exist a good correlation between the magnitude of second-phase response to a given insulin stimulator and the ability of the same stimulator to generate TDP. This was indeed the case: in pancreases stimulated with a range of glucose concentrations, the magnitude of TDP (measured by repetitive pulses) was closely correlated ($r^2 = 0.8$) with the rising slope of second-phase insulin release (9). Also, the fact that second-phase insulin response to glucose is often retained long after the disappearance of first-phase insulin release in early type 2 diabetic patients, in whom TDP seems intact, further supports this hypothesis.

Modeling the insulin response to glucose in humans. Is the above model applicable to insulin secretion in humans? Both TDI and TDP were demonstrated in humans, the former using glucose as well as nonnutrient secretagogues. Therefore, based on the ideas developed above, a mathematical model was constructed whose primary aim was to obtain information on the quantitative aspects of TDI, TDP, and the sensitivity of the β -cell to the acute glucose signal, from simulations of glucose infusion tests in individual subjects. Figure 7A presents a diagram of the model (it is outside the scope of this review to describe the mathematics involved, nor will the part of the closed-loop model which deals with the effect of endogenous insulin on glucose metabolism be dealt with; the reader is referred to the original publications [21,22]). This analysis was applied to a large number of glucose infusion tests in several clinical investigations in an attempt to describe in detail the individual insulin secretory characteristics (38–41). The validity of the model as well as its basic concept were tested in subgroups of subjects selected according to their simulation parameters during a standard glucose infusion test. For example, when subjects, in whom a high versus a low TDP parameter was obtained by simulation, were subjected to two consecutive

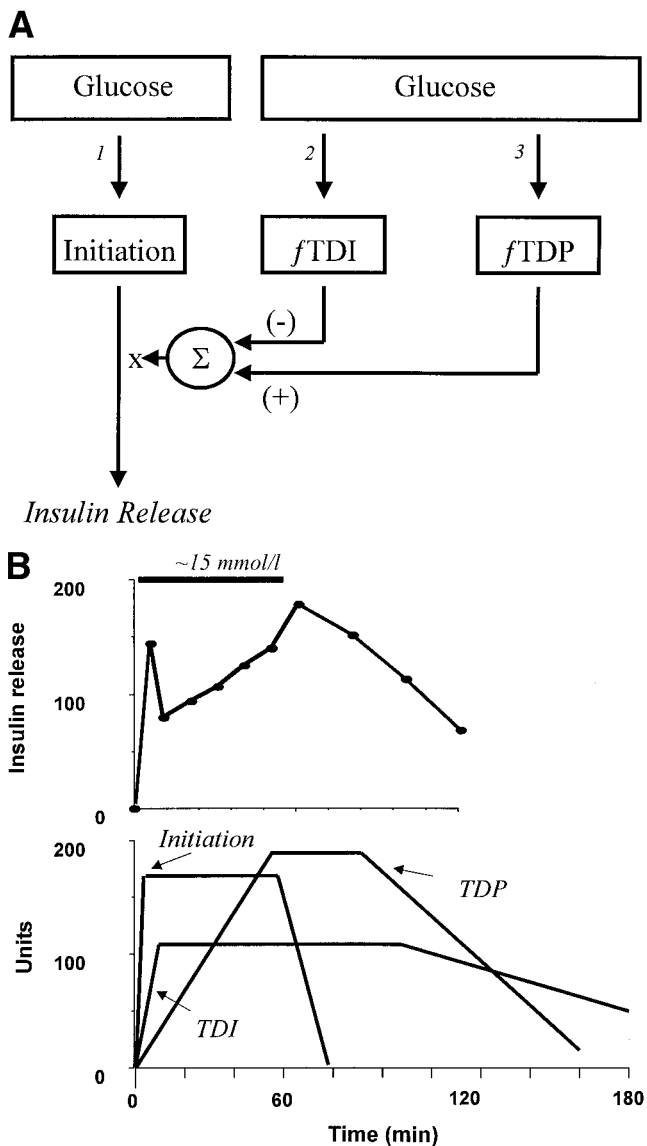


FIG. 7. A: Signal-modulation model of insulin release. It is postulated that substrates such as glucose generate three simultaneous actions in the β -cell (see text for details). TDI and TDP messages are integrated (circle) and modulate the direct insulinogenic signal of glucose in a multiplicative manner. **B:** Time-courses of the above β -cell signals during a ~ 15 -mmol/l glucose square-wave stimulus of 60 min. The insulin secretion initiatory signal of glucose is thought to follow relatively closely the changes in extracellular glucose, whereas TDI and TDP require more time to build up. The balance between the negative TDI signal and the positive TDP signal modulates the release rate to give it the classical biphasic pattern. Limited experimental evidence suggests that TDI may have a longer duration than that of TDP after withdrawal of the stimulus.

glucose challenges, the insulin response to the second stimulus was indeed amplified in subjects with high TDP values, whereas it failed to be in those with low TDP (21). The ability of the model to mimic TDI was less successful; nevertheless, the simulated TDI levels do represent a characteristic of the individual's insulin responsiveness, as indicated by the differences in the shape of the insulin response to glucose (21). It is obvious that, as additional biochemical and physiological information on the kinetics of insulin secretion in humans becomes available, such models can be refined and their ability to simulate the insulin response of normal as well as type 2 diabetic

subjects can be improved and applied to a variety of physiological and pharmacological secretagogues. From such analyses, it should be possible to infer key physiological parameters of β -cell function.

CONCLUSIONS

Substantial evidence indicates that insulin secretagogues, and mainly glucose, initiate a chain of events in the β -cell that act in parallel to control the rate of insulin release. Each of these events seems to have its own time course and glucose (and other secretagogue) dose dependence; their summation in the β -cell produces the observed insulin response. This is schematized in the model of Fig. 7B. Thus, during a square-wave stimulation with glucose, the hexose elicits a very rapid read-out signal (depolarization of the β -cell and Ca^{2+} inflow?), which is responsible for the ascending limb and peak of first-phase insulin response; this effect would persist throughout the stimulation as a sine qua non condition for insulin release. Shortly after this initial effect of glucose, TDI is switched on, forcing the insulin secretion rate to drop toward the interphase nadir. TDI also would be generated as long as the secretagogue is present; however, in the case of nutrient secretagogues, the TDP message building up in the β -cell would amplify the output, the insulin secretion rate rising toward the second-phase response (for a discussion of which β -cellular mechanisms could be candidates for TDP, see several articles in this supplement). The model also predicts that, at any given time, the acute responsiveness of the β -cell to a stimulation would be modulated by its past stimulations, i.e., by β -cell memories, the balance between TDI and TDP determining the magnitude of the insulin response.

Clearly, identifying the molecular events that control the β -cell signals that produce the unique dynamics of insulin release summarized in this review remains a major challenge. We do urge, nevertheless, that these complex interactions be kept in mind when studying specific biochemical/molecular events in the β -cell.

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REFERENCES

1. Blackard WG, Nelson NC: Portal and peripheral vein immunoreactive insulin concentrations before and after glucose infusion. *Diabetes* 19:302-306, 1970
2. Cerasi E, Hallberg D, Luft R: Simultaneous determination of insulin in brachial and portal veins during glucose infusion in normal and prediabetic subjects. *Horm Metab Res* 2:302-303, 1970
3. Horwitz DL, Starr JL, Mako ME, Blackard WG, Rubenstein AH: Proinsulin, insulin, and C-peptide concentrations in human portal and peripheral blood. *J Clin Invest* 55:1278-1283, 1975
4. Cerasi E: Aetiology of type II diabetes. In *Insulin: Molecular Biology to Pathology*. Ashcroft FM, Ashcroft SJH, Eds. Oxford, UK, Oxford University Press, 1992, p. 347-392

5. Palti Y, David GB, Lachov E, Mida YH, Schatzberger R: Islets of Langerhans generate wavelike electric activity modulated by glucose concentration. *Diabetes* 45:595–601, 1996
6. Pipeleers DG: Heterogeneity in pancreatic beta-cell population. *Diabetes* 41:777–781, 1992
7. Pipeleers D, Kiekens R, Ling Z, Wilikens A, Schuit F: Physiologic relevance of heterogeneity in the pancreatic beta-cell population. *Diabetologia* 37 (Suppl. 2):S57–S64, 1994
8. Jonkers FC, Henquin JC: Measurements of cytoplasmic Ca^{2+} in islet cell clusters show that glucose rapidly recruits beta-cells and gradually increases the individual cell response. *Diabetes* 50:540–550, 2001
9. Neshet R, Cerasi E: Biphasic insulin release as the expression of combined inhibitory and potentiating effects of glucose. *Endocrinology* 121:1017–1024, 1987
10. Cerasi E, Luft R, Efendic S: Decreased sensitivity of the pancreatic beta cells to glucose in prediabetic and diabetic subjects: a glucose dose-response study. *Diabetes* 21:224–234, 1972
11. Gutzeit A, Rabinovitch A, Karakash C, Stauffacher W, Renold AE, Cerasi E: Evidence for decreased sensitivity to glucose of isolated islets from spiny mice (*Acomys cahirinus*). *Diabetologia* 10:661–665, 1974
12. Cerasi E, Luft R: Plasma insulin response to sustained hyperglycaemia induced by glucose infusion in human subjects. *Lancet* 2:1359–1361, 1963
13. Curry DL, Bennett LL, Grodsky GM: Dynamics of insulin secretion by the perfused rat pancreas. *Endocrinology* 83:572–584, 1968
14. Grodsky GM, Curry D, Landahl H, Bennett L: [Further studies on the dynamic aspects of insulin release in vitro with evidence for a two-compartmental storage system] (multilingual). *Acta Diabetol Lat* 6 (Suppl. 1):554–578, 1969
15. Grodsky GM: A threshold distribution hypothesis for packet storage of insulin and its mathematical modeling. *J Clin Invest* 51:2047–2059, 1972
16. O'Connor MD, Landahl H, Grodsky GM: Comparison of storage- and signal-limited models of pancreatic insulin secretion. *Am J Physiol* 238: R378–R389, 1980
17. Cerasi E: An analogue computer model for the insulin response to glucose infusion. *Acta Endocrinol (Copenh)* 55:163–183, 1967
18. Daniel S, Noda M, Straub SG, Sharp GW: Identification of the docked granule pool responsible for the first phase of glucose-stimulated insulin secretion. *Diabetes* 48:1686–1690, 1999
19. Licko V: Threshold secretory mechanism: a model of derivative element in biological control. *Bull Math Biol* 35:51–58, 1973
20. Cerasi E: An analogue computer model for the insulin response to glucose infusion. *Simulation* 16:273, 1971
21. Cerasi E, Fick G, Rudemo M: A mathematical model for the glucose induced insulin release in man. *Eur J Clin Invest* 4:267–278, 1974
22. Cerasi E: Differential actions of glucose on insulin release: reevaluation of a mathematical model. In *Carbohydrate Metabolism*. Cobelli C, Bergman RN, Eds. New York, John Wiley & Sons, 1981, p. 3–24
23. Cerasi E: Feed-back inhibition of insulin secretion in subjects with high and low insulin response to glucose. *Diabete Metab* 1:73–76, 1975
24. Cerasi E: Potentiation of insulin release by glucose in man. I. Quantitative analysis of the enhancement of glucose-induced insulin secretion by pretreatment with glucose in normal subjects. *Acta Endocrinol (Copenh)* 79:483–501, 1975
25. Cerasi E: Potentiation of insulin release by glucose in man. II. Role of the insulin response, and enhancement of stimuli other than glucose. *Acta Endocrinol (Copenh)* 79:502–510, 1975
26. Cerasi E: Potentiation of insulin release by glucose in man. III. Different sensitivities of the pancreas to the initiating and potentiating actions of glucose in diabetic and prediabetic subjects. *Acta Endocrinol (Copenh)* 79:511–534, 1975
27. Neshet R, Tuch B, Hage C, Levy J, Cerasi E: Time-dependent inhibition of insulin release: suppression of the arginine effect by hyperglycaemia. *Diabetologia* 26:142–145, 1984
28. Cerasi E, Neshet R: Assessment of insulin secretion dynamics. In *Methods in Diabetes Research*. Clarke WL, Lerner J, Pohl SL, Eds. New York, John Wiley & Sons, 1986, p. 77–90
29. Neshet R, Eylon L, Segal N, Cerasi E: Beta-cell memory to insulin secretagogues: characterization of the time-dependent inhibitory control system in the isolated rat pancreas. *Endocrinology* 124:142–148, 1989
30. Prentki M, Matschinsky FM: Ca^{2+} , cAMP, and phospholipid-derived messengers in coupling mechanisms of insulin secretion. *Physiol Rev* 67:1185–1248, 1987
31. Zawulich WS, Rasmussen H: Control of insulin secretion: a model involving Ca^{2+} , cAMP and diacylglycerol. *Mol Cell Endocrinol* 70:119–137, 1990
32. Efendic S, Lins PE, Cerasi E: Potentiation and inhibition of insulin release in man following priming with glucose and with arginine: effect of somatostatin. *Acta Endocrinol (Copenh)* 90:259–271, 1979
33. Neshet R, Waldman L, Cerasi E: Time-dependent inhibition of insulin release: glucose-arginine interactions in the perfused rat pancreas. *Diabetologia* 26:146–149, 1984
34. Neshet R, Abramovitch E, Cerasi E: Correction of diabetic pattern of insulin release from islets of the spiny mouse (*Acomys cahirinus*) by glucose priming in vitro. *Diabetologia* 28:233–236, 1985
35. Grill V, Adamson U, Cerasi E: Immediate and time-dependent effects of glucose on insulin release from rat pancreatic tissue: evidence for different mechanisms of action. *J Clin Invest* 61:1034–1043, 1978
36. Grill V, Adamson U, Rundfeldt M, Andersson S, Cerasi E: Glucose memory of pancreatic B and A2 cells: evidence for common time-dependent actions of glucose on insulin and glucagon secretion in the perfused rat pancreas. *J Clin Invest* 64:700–707, 1979
37. Borg LA, Westberg M, Grill V: The priming effect of glucose on insulin release does not involve redistribution of secretory granules within the pancreatic B-cell. *Mol Cell Endocrinol* 56:219–225, 1988
38. Efendic S, Cerasi E, Elander I, Thornqvist C, Fick G, Berghlund B, Luft R: Studies on low insulin responders: glucose tolerance and insulin response to intravenous and oral glucose challenges in 226 normal subjects and 25 subjects with mild or chemical diabetes. *Acta Endocrinol Suppl* 224:5–32, 1979
39. Efendic S, Wajngot A, Cerasi E, Luft R: Insulin release, insulin sensitivity, and glucose intolerance. *Proc Natl Acad Sci U S A* 77:7425–7429, 1980
40. Iselius L, Lindsten J, Morton NE, Efendic S, Cerasi E, Haegermark A, Luft R: Genetic regulation of the kinetics of glucose-induced insulin release in man: studies in families with diabetic and non-diabetic probands. *Clin Genet* 28:8–15, 1985
41. Alvarsson M, Grill VE, Wajngot A, Cerasi E, Efendic S: Long-term stability of low insulin responses to glucose in non-diabetic subjects. *Acta Endocrinol (Copenh)* 127:33–37, 1992