First-phase insulin secretion: does it exist in real life? 
Considerations on shape and function

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Caumo, Andrea, and Livio Luzi. First-phase insulin secretion: does it exist in real life? Considerations on shape and function. Am J Physiol Endocrinol Metab 287: E371–E385, 2004; 10.1152/ajpendo.00139.2003.—To fulfill its preeminent function of regulating glucose metabolism, insulin secretion must not only be quantitatively appropriate but also have qualitative, dynamic properties that optimize insulin action on target tissues. This review focuses on the importance of the first-phase insulin secretion to glucose metabolism and attempts to illustrate the relationships between the first-phase insulin response to an intravenous glucose challenge and the early insulin response following glucose ingestion. A clear-cut first phase occurs only when the β-cell is exposed to a rapidly changing glucose stimulus, like the one induced by a brisk intravenous glucose administration. In contrast, peripheral insulin concentration following glucose ingestion does not bear any clear sign of biphasic shape. Coupling data from the literature with the results of a β-cell model simulation, a close relationship between the first-phase insulin response to intravenous glucose and the early insulin response to glucose ingestion emerges. It appears that the same ability of the β-cell to produce a pronounced first phase in response to an intravenous glucose challenge can generate a rapidly increasing early phase in response to the blood glucose profile following glucose ingestion. This early insulin response to glucose is enhanced by the concomitant action of incretins and neural responses to nutrient ingestion. Thus, under physiological circumstances, the key feature of the early insulin response seems to be the ability to generate a rapidly increasing insulin profile. This notion is corroborated by recent experimental evidence that the early insulin response, when assessed at the portal level with a frequent sampling, displays a pulsatile nature. Thus, even though the classical first phase does not exist under physiological conditions, the oscillatory behavior identified at the portal level does serve the purpose of rapidly exposing the liver to elevated insulin levels that, also in virtue of their up-and-down pattern, are particularly effective in restraining hepatic glucose production.

in vivo physiology? What is the relationship between the first-phase insulin response to a brisk intravenous glucose challenge and the early insulin response following glucose ingestion? What are the effects of such insulin responses on glucose metabolism?

Before these issues are addressed, a precise definition of first-phase insulin secretion is required. For the purpose of the present review, five different modes (or phases) of insulin secretion can be identified: 1) basal insulin secretion is the way insulin is released in the postabsorptive state; 2) the cephalic phase of insulin secretion is evoked by the sight, smell, and taste of food (before any nutrient is absorbed by the gut) and is mediated by pancreatic innervation (2, 63); 3) first-phase insulin secretion is defined as the initial burst of insulin, which rises more gradually and is directly related to the degree and duration of the stimulus; 4) after the acute response, there is a second-phase insulin secretion, which rises more gradually and is directly related to the degree and duration of the stimulus; 5) finally, a third phase of insulin secretion has been described, albeit only in vitro (49). During all these stages, insulin is secreted, like many other hormones, in a pulsatile fashion, resulting in oscillatory concentrations in peripheral blood. Oscillations include rapid pulses (recurring every 8–15 min) superimposed on slower, ultradian oscillations.

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Fig. 1. Differences between the incremental (above basal) plasma insulin concentration profiles in response either to an intravenous glucose infusion producing a square wave of hyperglycemia (A) or to a meal (B). The response to the intravenous glucose challenge shows a biphasic profile, with distinct 1st- (0–10 min) and 2nd-phase (10–30 min) insulin-secretory responses. In contrast, the insulin response to the physiological entry of glucose does not exhibit a clearly distinguishable biphasic pattern. Of note is that the insulin response observed after food ingestion cannot be accounted for solely by the associated changes in the blood glucose level but also depends on other factors, such as the presence of free fatty acids and other secretagogues in the meal, the neurally activated cephalic phase, and gastrointestinal hormones.

Insulin secretion can be induced by other energetic substrates besides glucose (particularly amino acids), as well as by hormones and drugs. In the following review, we will focus on the insulin response to glucose. Of crucial importance to the present review is to make a clear distinction between the insulin response following an intravenous glucose challenge and that following glucose or food ingestion. The intravenous administration of glucose triggers a biphasic insulin response (featuring a rapid increase with a peak, an interpeak nadir, and a subsequent slower increasing phase) only when glucose (above basal) plasma insulin concentration profiles are recorded in response to a square wave of hyperglycemia. A “slow-ramp” glucose input induces gradually larger secretion without a well-defined first phase (48, 107). More importantly, a well-defined first phase is lacking under physiological conditions, i.e., when glucose is given orally. Thus, in the course of this review, we will refer to first-phase insulin secretion only in the context of “rapid” intravenous glucose stimuli and will denote as early phase the insulin response observed during the initial 30 min that follow glucose ingestion (Fig. 1).

The review is organized as follows. First, we will briefly describe the methods used to quantify insulin secretion in vivo. Then, we will summarize investigations assessing first-phase insulin secretion in response to glucose stimuli in vitro and in vivo and the most significant modeling attempts that have been put forth to better understand the mechanisms underlying biphasic insulin secretion. We will then provide an essential compendium of the studies that investigated the impact of first-phase insulin secretion on glucose metabolism. Subsequently, we will tackle insulin secretion under physiological conditions and try to elucidate the relationships between the initial insulin response to intravenous glucose (first phase) and oral glucose (early phase) through the use of a model simulation. After that, we will move on to summarize the beneficial effects that the early insulin secretion exerts on prandial glucose metabolism. Finally, we will briefly highlight recent studies showing that the early insulin response observed in peripheral blood after glucose ingestion is the dampened version of a portal oscillatory pattern that repeatedly exposes the liver to a first-phase-like insulin pattern.

MEASUREMENT OF INSULIN SECRETION

In assessing insulin secretion in vivo, one faces several difficulties and must use mathematical models as an aid (as reviewed in Refs. 57, 71, and 114). Insulin is secreted by the β-cell into the portal vein, which is usually not accessible. In addition, plasma insulin levels reflect not only insulin secretion but also insulin distribution and degradation (i.e., the kinetics). Finally, before diluting into the peripheral circulation, insulin passes through the liver, where it undergoes a large (~50%) and probably time-varying extraction (45). The latter shortcoming can be bypassed by exploiting the properties of C-peptide. C-peptide is cosecreted with insulin on an equimolar basis but, unlike insulin, is not extracted by the liver (Fig. 2). The gold standard to derive C-peptide secretion from C-peptide concentrations is based on C-peptide kinetic modeling and deconvolution (the so-called Eaton-Polonsky approach (37, 87)). Deconvolution is a procedure that allows one to infer the

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**Fig. 2.** Schematic representation of the secretion and kinetics of insulin and C-peptide. The β-cell releases equimolar amounts of insulin (top) and C-peptide (bottom). Part of released insulin is degraded by the liver, but all of the released C-peptide reaches the periphery, where both peptides are measured in plasma. Both insulin and C-peptide follow 2-compartment kinetics, although 1-compartment descriptions are frequently used in the literature (especially for insulin, as discussed in Ref. 44). This general schema is at the basis of various modeling approaches to the estimation of insulin secretion in vivo.
secretion rate of a substance from its peripheral concentrations by mathematically removing the impact of the substance kinetics. To estimate C-peptide secretion by deconvolution, knowledge of C-peptide kinetics is thus required. C-peptide kinetics are usually determined in a separate experiment by analyzing the C-peptide decay curve following a bolus injection of biosynthetic C-peptide. If C-peptide kinetics cannot be obtained in a separate experiment, the population-based C-peptide kinetic parameters reported by Van Cauter et al. (118) can be used as a reliable alternative for all those situations where renal function, and thus C-peptide kinetics, are not impaired. Deconvolution has been extensively used to reconstruct insulin secretion in various experimental conditions (14, 37, 56, 87, 103). Recent advances in the deconvolution techniques (85, 108) allow us to cope better with the highly dynamic nature of the first-phase insulin response.

A simpler alternative to deconvolution, denoted the “combined model,” has been introduced by Volund et al. (119) and subsequently applied and refined by Watanabe and colleagues (121, 122). The combined model allows for the estimation of insulin secretion without separate experimental assessment or a priori assumption of the C-peptide kinetic parameters. This is accomplished by simultaneously modeling C-peptide and insulin kinetics and exploiting the fact that C-peptide and insulin data are two sources of information concerning the same secretion rate (as shown in Fig. 2). The combined model has been validated against deconvolution under experimental conditions mimicking the slow changes in insulin secretion observed during an oral glucose tolerance test (OGTT) (62). Another approach to measure insulin secretion is the minimal model of Toffolo et al. (115) (“minimal” denotes here an optimal degree of complexity). The minimal model uses only C-peptide data and individualizes C-peptide kinetics using the parameters of Van Cauter et al. (118). Its distinctive and appealing feature is an explicit description of glucose control on β-cell secretion. This allows the model to provide not only insulin secretion but also indexes of β-cell responsivity to glucose that can be helpful for the characterization of impaired mechanisms of insulin secretion in pathophysiological states. The model was originally developed to interpret the intravenous glucose tolerance test (IVGTT) (115), but it has been recently adapted to other experimental conditions such as up-and-down glucose staircase profiles (113) and the OGTT (13). The model has been validated against deconvolution in experimental protocols characterized by slow changes in insulin secretion (e.g., up-and-down staircase profiles) (14). Other β-cell models that attempt to describe the functional dependence of insulin secretion on glucose concentration have been developed in recent years, especially to quantify insulin secretion during experimental protocols reproducing normal life conditions (e.g., mixed-meal tests and OGTTs) (29, 56, 72). These models, which differ for the specific assumptions about the control exerted by glucose on β-cell secretion, have been recently reviewed (81).

Although the quantitative assessment of insulin secretion is usually based on the measurement of C-peptide, it must be recognized that, from a qualitative standpoint, the profile of plasma insulin concentration is better than that of C-peptide in reproducing the shape of insulin secretion (104). This occurs because the half-life of insulin (~10 min) (44) is markedly shorter than that of C-peptide (~35 min) (118). Thus alternative approaches, which simply rely on insulin concentrations, to estimating surrogate indexes of first- and second-phase insulin secretion, have been developed. For instance, insulin secretion indexes can be derived from a hyperglycemic clamp by calculating areas under the insulin concentration curve (from 0 to 10 min for the first phase, and from 10 min onward for the second phase). Another widely used index of first-phase insulin secretion is the acute insulin-secretory response to glucose (AIRGlc), which is calculated as the mean incremental increase in plasma insulin concentration in samples obtained at 3, 4, and 5 min of an IVGTT (25). A more sophisticated approach to the estimation of indexes of β-cell function from IVGTT insulin data is based on the minimal-model description of the functional relationship between the plasma glucose concentration and the posthepatic insulin delivery rate (112).

In studying the insulin response to an oral glucose load, first and second phases are not clearly distinguishable. During a meal, a typical index of early insulin response is the insulin increment at 30 min (ΔI30). When this index is normalized to the corresponding glucose increment (ΔI30/ΔGlc30), it takes the name insulinogenic index and measures a sort of sensitivity of the β-cell to glucose (84).

**BIPHASIC NATURE OF THE INSULIN RESPONSE: IN VITRO AND IN VIVO STUDIES**

The presence of a biphasic dynamics of β-cell release in response to glucose has been extensively investigated in vitro, both at the level of the islets comprising the endocrine pancreas and at the organ level. The physiology of insulin secretion at the islet level involves several molecular events whose description goes beyond the scope of this review. We refer the reader interested in having an up-to-date overview of insulin secretion at the islet level to a recent collection of review articles appearing in a monographic issue of the journal *Diabetes* dedicated to insulin secretion (*Diabetes* 51, Suppl 1, 2002). Insulin secretion at the organ level was extensively studied by Grodsky and colleagues (an overview of their findings can be found in Ref. 50). They used the isolated rat pancreas perfused with a buffer containing glucose to study insulin secretion dynamics in response to a variety of glucose stimuli, such as stepwise and staircase increments as well as short pulses (30, 48, 51, 52). These studies showed that the β-cell responds to a prolonged single step of glucose with a rapid and transient first phase of insulin secretion followed by a slower but increasing-in-time second phase (Fig. 3). The magnitude of the first phase in response to a single-step glucose stimulus increases with increasing doses of glucose. Specifically, the amount of insulin released during the first phase is a sigmoidal function of glucose concentration (with a half-maximum for glucose of ~135 mg/dl). When the glucose stimulus is repeated in short intervals, the first-phase insulin response is inhibited; in contrast, if longer time intervals are used, enhancement of the first-phase insulin response is observed at the second stimulation. Thus the magnitude of the first phase is dependent on the pancreas’s past history of stimulation by glucose. The first phase takes place almost instantaneously after the beginning of the stimulation, and, at any glucose level, the rapid rise and decay of the first phase occur within 3–4 min. The second phase can last several hours if the β-cell is continuously exposed to glucose. A desensitization phase, characterized by...
a sustained but lower insulin release, can be demonstrated for up to 24 h with prolonged exposure to glucose. The detailed portrait of insulin secretion provided by Grodsky and coworkers using the perfused rat pancreas was corroborated by Bergman and Urquhart (9) using a peculiar experimental approach. These investigators devised a method, called the "pilot gland" approach, to perfuse the pancreas with whole blood rather than an artificial medium, thus exposing the organ to the metabolic factors that are normally present in vivo. With this approach, the pancreas extirpated from a small dog (i.e., the pilot gland) is perfused with the arterial blood of an intact, anesthetized, large dog. The weight ratio between the two dogs (1:7) is such that dynamic tests can be performed on the pilot pancreas without causing evident metabolic alterations in the large dog.

Phasic insulin secretion can be demonstrated in vivo by administering glucose intravenously in such way as to quickly increase plasma glucose concentration. Typical glucose stimuli are the bolus injection (whose clinical counterpart is the IVGTT), the primed constant infusion, and the primed variable infusion establishing a square wave of hyperglycemia [whose clinical counterpart is the hyperglycemic clamp (33)]. Such inputs expose the β-cell to an abrupt rise in glucose concentration and are able to elicit a biphasic insulin response (23, 24, 65, 94). Let us focus our attention on the advantages and drawbacks of the IVGTT compared with the hyperglycemic clamp. The IVGTT is the protocol of choice when simplicity is required, because the hyperglycemic clamp (whose features and applications have been reviewed in Ref. 40) entails a sophisticated experimental procedure, is labor intensive, and requires considerable expertise. On the other hand, one advantage of the hyperglycemic clamp over the IVGTT is that the pancreas is exposed to the same glucose increment for the desired duration, whereas during the IVGTT the glucose peak may differ among subjects and glucose concentration decays at a rate that depends on the individual’s glucose tolerance. In addition, the prolonged elevation of glucose concentration that the hyperglycemic clamp brings about allows a clearer determination of the second-phase insulin secretion with respect to the IVGTT. For these reasons, the hyperglycemic clamp is considered the gold standard method for the assessment of the biphasic insulin response in vivo. How fast does the pancreas respond to an intravenous glucose challenge? When blood can be sampled from the portal vein, the time lag between the rise of glucose concentration to the peak insulin response is short: 1–2 min. When only peripheral sampling is available, the biphasic insulin response is delayed and dampened due to the passage through the liver and distribution in the peripheral blood. For instance, during a hyperglycemic clamp (Fig. 4), the first phase occurs between 3 and 5 min; then the plasma insulin falls rapidly, reaching a nadir at 10 min; finally, a second phase is observed during which plasma insulin increases almost linearly as long as the glucose stimulus is maintained. The impairment of the first-phase insulin response to intravenous glucose is a marker of the pathology of the β-cell. For example, the first-phase insulin response is almost invariably reduced in
patients with impaired glucose tolerance (IGT) or in the early stages of type 2 diabetes, as shown in Refs. 24, 39, and 101 and reported in countless review articles (see, for instance, Refs. 32 and 120).

EXPLANATION OF THE BIPHASIC INSULIN RESPONSE: ROLE OF MODELS

An abundance of research has been performed to shed light on the mechanisms underlying the biphasic insulin response observed in an isolated and perfused pancreas or in an intact organism following abrupt changes in glucose concentration. Many studies have investigated the macrophysiology of β-cell secretion, whereas many others have tried to identify the basic biochemical and molecular processes that are involved. In the following, we will briefly deal with the former type of studies, and we refer the reader interested in having an up-to-date overview of insulin secretion at the molecular level to the aforementioned monographic issue of the journal Diabetes devoted to insulin secretion (Diabetes 51, Suppl 1, 2002). In the studies that have addressed the biphasic insulin secretion in terms of the macrophysiology of the event, mathematical modeling has played a relevant role. Two distinct modeling approaches have been developed over the years: the “storage-limited model” and the “signal-limited model” (comparisons of these two model categories can be found in Refs. 78 and 80).

The storage-limited model was developed by Grodsky and coworkers (48, 52) on the basis of experimental data of insulin secretion obtained in vitro from the isolated perfused pancreas [it should be noted that secretion data obtained in vivo in secretion obtained in vitro from the isolated perfused pancreas coworkers (48, 52) on the basis of experimental data of insulin these two model categories can be found in Refs. 78 and 80). The storage-limited model was developed by Grodsky and coworkers (48, 52) on the basis of experimental data of insulin secretion obtained in vitro from the isolated perfused pancreas it should be noted that secretion data obtained in vivo in humans are compatible with this kind of model (94)]. The storage-limited model assumes that the β-cell contains two distinct pools of insulin granules: a small (2%), labile pool accessible for immediate release [which has been anatomically distinct pools of insulin granules: a small (2%), labile pool accessible for immediate release (Fig. 6)] and a larger (99%), stable pool that feeds slowly into the labile pool. Glucose promotes the provision of new insulin and the transfer from the storage to the labile pool. According to this model, the transient nature of the first-phase insulin release is the consequence of the voiding of the labile pool early during glucose stimulation, whereas the exhaustion corresponds to the nadir between the two phases of insulin secretion. When sufficient time is allotted, the labile compartment is refilled by the transfer of granules from the large insulin pool. The refilling process is driven by glucose through a potentiating signal that accumulates gradually. With time, the potentiating signal causes the insulin content in the labile compartment to rise, thus producing the increasing second-phase secretion. One drawback of the original formulation of the storage-limited model (52) is that a constant size of the labile pool is not capable of accommodating for the observation that progressively higher concentrations of glucose produce first-phase responses of increasing amplitude. For this reason, the model was subsequently refined by incorporating the assumption that the labile pool is not homogeneous but contains populations of β-cells (packets) with different threshold sensitivities to glucose (48). These packets release their insulin content in an all-or-nothing fashion when their thresholds to glucose are exceeded (Fig. 5) [the theory of the threshold secretory mechanism was formalized by Licko (67)].

The signal-limited model was developed in a series of publications by Cerasi and coworkers (20–22, 77). These investigators abandoned the pool model in favor of the idea that biphasic insulin release is the result of the dynamic interaction between stimulatory and inhibitory events initiated by glucose, each of them having its own kinetics and dose dependence. In this model, it is assumed that high levels of glucose rapidly trigger a signal for the initiation of insulin release. This signal is thought to be proportional to the increment of glucose concentration above basal. In addition to this initiation, glucose enhances the sensitivity of the islet, a process denoted as potentiation, which increases the rate of insulin release triggered by the initiating action of glucose. This potentiation is assumed to be time varying and to have a relatively long half-life. Finally, it is postulated that glucose induces an inhibitory effect on insulin release, thereby reducing the stimulatory effects of initiation and potentiation. If the time course of this inhibition is intermediary to those of initiation and potentiation, the superposition of these three signals is able to produce the classical biphasic β-cell response with its distinctive features (Fig. 6, top). Based on this idea, a glucose-insulin model was constructed whose primary aim was to measure the quantitative aspects (time characteristics, dose dependencies, etc.) of initiation, potentiation, and inhibition from the analysis of individual stimulus-response experiments (22) (Fig. 6, bottom).

EFFECTS OF FIRST-PHASE INSULIN SECRETION ON GLUCOSE METABOLISM

Insulin plays a pivotal role in glucose homeostasis by virtue of its ability to inhibit hepatic glucose production and stimulate glucose uptake. The magnitude of these insulin effects depends on the concentration of insulin at the liver site and in the interstitial fluids, respectively. Figure 7 shows the results of a model simulation illustrating the stages that a biphasic insulin response goes through after being released by the β-cell. First, insulin passes through the liver, where it exerts some of its effects; then, it enters the general circulation where it is diluted; and finally, it is carried through the body and reaches the interstitial fluids, where it interacts with the cell receptors (the interstitial fluids are modeled as a compartment remote from plasma, in accord with Refs. 7, 105, and 123).

Anatomically, the liver is the most obvious candidate for being the target organ for first-phase insulin secretion. Because
Insulin is secreted directly into the portal system, this ensures a greater hepatic than peripheral insulinization (porto-systemic gradient). In addition, the shape of sinusoidal insulin closely reproduces the shape of insulin secretion. Thus any direct effect that insulin is able to exert on the liver is magnified by the presence of the transient but elevated insulin levels that characterize the first phase. Insulin concentration in the interstitial fluids does not resemble insulin secretion as a result of the delay associated with the transit of insulin through the endothelial barrier. In particular, interstitial insulin bears no trace of the original biphasic shape. Nevertheless, the availability of a large first-phase secretion has been shown to promote a quicker activation of glucose uptake in dogs (46). Thus it is likely that the first phase will have beneficial effects on virtually all the insulin-dependent processes that ensure glucose homeostasis. The overall result is an improvement of glucose tolerance, as shown in humans by Calles-Escandon and Robbins (18). In that study, the authors used a brief intravenous infusion of somatostatin to selectively inhibit the first-phase insulin response to an IVGTT in normal subjects (in this way, they acutely created a model to reproduce the loss of the first phase seen in the diabetic state). They found that the loss of the first-phase insulin response was associated with a reduced glucose tolerance and a possible hampering of the thermic effects of ingested carbohydrates.

In the following paragraphs, we will summarize the results of studies investigating the benefits associated with first-phase insulin release. We will first illustrate the effects of the first phase on peripheral tissues and then examine the effects of the first phase at the liver site.

Effects of first-phase insulin response on peripheral tissues. The time course of glucose uptake by peripheral tissues lags considerably following insulin response. It has been suggested that this delay is due to the time it takes insulin to cross the endothelial barrier and reach the interstitial medium bathing insulin is secreted directly into the portal system, this ensures a greater hepatic than peripheral insulinization (porto-systemic gradient). In addition, the shape of sinusoidal insulin closely reproduces the shape of insulin secretion. Thus any direct effect that insulin is able to exert on the liver is magnified by the presence of the transient but elevated insulin levels that characterize the first phase. Insulin concentration in the interstitial fluids does not resemble insulin secretion as a result of the delay associated with the transit of insulin through the endothelial barrier. In particular, interstitial insulin bears no trace of the original biphasic shape. Nevertheless, the availability of a large first-phase secretion has been shown to promote a quicker activation of glucose uptake in dogs (46). Thus it is likely that the first phase will have beneficial effects on virtually all the insulin-dependent processes that ensure glucose homeostasis. The overall result is an improvement of glucose tolerance, as shown in humans by Calles-Escandon and Robbins (18). In that study, the authors used a brief intravenous infusion of somatostatin to selectively inhibit the first-phase insulin response to an IVGTT in normal subjects (in this way, they acutely created a model to reproduce the loss of the first phase seen in the diabetic state). They found that the loss of the first-phase insulin response was associated with a reduced glucose tolerance and a possible hampering of the thermic effects of ingested carbohydrates.

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the insulin-sensitive cells (10, 123). In particular, it has been shown in dogs that changes in glucose uptake are closely predicted by changes in hindlimb lymph insulin (representing interstitial insulin bathing the skeletal muscle) (95). Thus, to appreciate the effect of the biphasic insulin response on glucose uptake, one should make reference to the time course of insulin in the interstitial fluids, not in plasma. As insulin reaches the interstitial fluids, the biphasic pattern is no longer appreciable, because the first phase is considerably attenuated and merged with the second phase (Fig. 7). Even though insulin loses its biphasic pattern when it reaches the interstitial fluids, a large first-phase insulin secretion causes remote insulin to increase more quickly than it otherwise would. Getty et al. (46) have shown in dogs that, when the contribution of the first-phase insulin response to the overall increase in insulin is of the order of \( \geq 50\% \), this produces a rise in insulin lymph concentration that is much more rapid than the one observed when only the second phase is present. This, in turn, promotes a quick activation of glucose uptake by the tissues.

Because first-phase insulin secretion determines a time course of interstitial insulin concentration that has beneficial effects on glucose uptake, it is not unreasonable to hypothesize that it may also promote a quick activation of insulin’s indirect effect on hepatic glucose production. It has been established that insulin’s inhibitory effect on hepatic glucose production is in part direct, i.e., due to the interaction between hepatic sinusoidal insulin with the hepatocyte, and in part indirect, i.e., due to the action of insulin on extrahepatic tissues, the latter action consisting mainly of the antilipolytic effect of insulin on adipose tissue (1, 26, 47, 66, 70, 96, 98). The insulin-induced decrease of substrate flow to the liver and the subsequent inhibition of hepatic glucose production [i.e., the pillar of the so-called single-gateway hypothesis (6)] is a rather slow process. It has been suggested that the delay in insulin suppression of lipolysis may be secondary to the delay in insulin reaching the interstitial fluids. Thus it is likely that insulin concentration in the interstitial fluids plays a crucial role also in regulating insulin’s indirect inhibition of hepatic glucose production. Because the first-phase insulin response favors a more rapid elevation of insulin concentration in the interstitial fluids, as ascertained by Getty et al. (46), it is plausible that insulin’s indirect inhibition of hepatic glucose production is also activated more rapidly when the first-phase insulin response is present.

**Effects of first-phase insulin response on the liver.** Insulin has a direct inhibitory effect on hepatic glucose production. In dog studies, insulin’s direct effect on hepatic glucose production has been found to be potent and more important that insulin’s indirect effect, at least in nondiabetic animals (26). Lewis et al. (66) have shown in humans that hepatic glucose production is inhibited to a greater extent with portal compared with peripheral insulin delivery, with matched peripheral insulin levels. Maheux et al. (70) have shown in humans that an increase in portal vein insulin concentration can rapidly inhibit hepatic glucose production independently of an increase in peripheral plasma insulin concentration. Thus, by acutely elevating liver sinusoidal insulin concentration, first-phase insulin secretion brings about a rapid inhibitory effect on hepatic glucose production. In addition, when the delayed version of the first phase reaches the interstitial fluids, the above-mentioned indirect effects of insulin will also become manifest and contribute to restrain hepatic glucose production. The relative importance and the precise timing of either the direct or indirect effects of first-phase insulin secretion on hepatic glucose production have not been fully elucidated yet, but experimental evidence has shown that the overall result on hepatic glucose production is remarkable and long lasting. In collaboration with Dr. Ralph DeFronzo, one of us was the first to document the crucial role played by first-phase insulin secretion in restraining hepatic glucose production (68). In that study, normal young subjects underwent hyperglycemic clamp studies in which somatostatin was administered, basal glucose and insulin levels were replaced, and exogenous insulin was infused to mimic either a normal (first- and second-phase) or a second-phase-only insulin response (Fig. 8, A and C). We found that the presence of the first phase was associated with
a more rapid and marked suppression of hepatic glucose production (Fig. 8D) (a finding confirmed with a similar experimental approach in Ref. 41), whereas glucose clearance (Fig. 8D) was not significantly affected (as previously shown in Ref. 12 with the euglycemic hyperinsulinenic clamp). Interestingly, despite the transient nature of the first phase, in our study the enhancement of the inhibition of hepatic glucose production was still present 2 h after the beginning of the test. This suggests that priming the liver with insulin may produce long-term benefits, a notion that is confirmed under prandial conditions, as we will see in the following sections of this review.

Another way in which the first-phase insulin response can act on the liver is through the interaction with glucagon [see, in this regard, the review by Unger (116)]. Steiner et al. (109) carried out experiments in dogs to investigate the ability of the individual components of the biphasic insulin response to counteract glucagon-stimulated hepatic glucose production. They infused glucagon intraportally to determine a fourfold increase in plasma glucagon and observed the effects on glucose metabolism under four conditions: 1) in the absence of an incremental insulin release, 2) in the presence of a simulated first-phase insulin release, 3) in the presence of a simulated second-phase insulin release, and 4) in the presence of a simulated first- and second-phase insulin release. With this experimental approach, the investigators showed that both phases of insulin release are important for full counterregulation of the action of glucagon on glucose metabolism. When the first-phase insulin response is lacking, the insulin-to-glucagon ratio is rapidly altered in favor of glucagon, thus leading to increased hepatic glucose production and hyperglycemia. Restoration of the first-phase insulin profile in plasma, even in the absence of the second phase, curbs the glucagon-mediated rise in plasma glucose concentration. In a subsequent study, the same group of investigators showed that first-phase insulin response acts primarily by inhibiting hepatic glucose production through a reduction in gluconeogenesis (110).

INSULIN SECRETION UNDER PHYSIOLOGICAL CONDITIONS

We have seen that a clear-cut first-phase insulin response can be elicited in vivo resorting only to stimuli that rapidly elevate blood glucose concentration. Such stimuli, however, are not physiological, because abrupt and marked increases in blood glucose concentration do not occur in nature. Under physiological conditions, after a meal for instance, glucose concentration increases gradually, and the insulin response measured in the peripheral blood does not bear any clear sign of a biphasic shape.

What is the relationship between the biphasic insulin response following an intravenous glucose stimulus and the early insulin response, much less biphasic, or not biphasic at all, observed under physiological conditions? In the attempt to answer this question, it may be of some help to consider the biphasic nature of insulin secretion from a teleological perspective. A biphasic insulin response can be conceived as the superposition of a priming bolus and a secondary infusion of insulin, this combination allowing the rapid achievement of elevated insulin concentrations. It is plausible that the same β-cell dynamic properties that are able to generate a biphasic secretion in response to a brisk, intravenous glucose challenge are still operating in response to the gradual entry of glucose from the gut. Thus, even though insulin secretion tends to lose its biphasic shape under physiological conditions, the early insulin response may still be aimed to quickly shift glucose metabolism from the fasting to the prandial state.

To gain some quantitative insight into this issue, we performed a simulation of glucose-stimulated β-cell secretion using the minimal model of Toffolo et al. (113). This model (illustrated in Fig. 9) postulates that insulin secretion in response to glucose elevation is the sum of two components, one static and the other dynamic. The static component (governed by parameters α and β) is proportional to Δglucose concentration (it should be noted that a delay between the glucose stimulation and the actual insulin release by the β-cell is assumed). The dynamic component (governed by parameter kd) is proportional to the derivative of glucose concentration and accounts for the observation that rapid changes in glucose concentration enhance insulin secretion (25, 79). Coupling this β-cell model with a two-compartment model of insulin kinetics (whose parameters were taken from Ref. 87) and assuming a 50% first-pass hepatic extraction of insulin, we were able to predict the β-cell secretion and the corresponding profile of peripheral insulin concentration in response to two different glucose stimuli. The two glucose stimuli were intravenous glucose infusions producing either a square wave of hyperglycemia or a gradual glycemic elevation mimicking the postprandial hyperglycemia (in the following, the latter glucose input will be denoted meal-like study). The model operated under conditions reproducing either a normally functioning β-cell or a defective β-cell with a selective impairment of the first-phase insulin response. To reproduce a normally functioning β-cell, we fixed the parameters of the model (α, β, kd) to the values that are found in normal subjects in response to intravenous glucose administration. To reproduce a β-cell with a defective first phase, we left unchanged the parameters α and β of the static control but assumed a 66% reduction in parameter kd of

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**Fig. 9.** Schematic representation of the model of glucose-stimulated β-cell secretion developed by Toffolo et al. (113) and subsequently applied to the oral glucose tolerance test (OGTT) by Breda et al. (13). The model includes a static control, i.e., a secretion component that is a function of plasma glucose concentration, and a dynamic control, i.e., a secretion component that is proportional to the positive values of the glucose concentration derivative. The static component is governed by parameters α and β, whereas the derivative component is governed by parameter kd. Parameter α represents the dynamic delay between the elevation of glucose concentration and the release of insulin by the pancreas. Parameter β is the proportionality constant between glucose concentration and the static component of the secretion response. Parameter kd is the proportionality constant between the rate of increase of glucose and the derivative component of the secretion response.
the dynamic control. The results of the simulation are shown in Fig. 10. Top, shows the glucose profiles following either a square-wave (left) or a meal-like (right) study. Figure 10, middle, shows the corresponding model predictions of insulin secretion under the assumptions of a normal or a defective β-cell. Below, in Fig. 10, bottom, are the corresponding profiles of peripheral insulin concentration. The results deserve some comments. It can be seen that the insulin response to the square wave of hyperglycemia is similar to the one actually measured during a hyperglycemic clamp (Fig. 10, middle and bottom left). The model predicts a biphasic secretory pattern, with a peak of insulin concentration at 3 min, an interpeak nadir at ~10 min, and a subsequent increase attaining a plateau. The pattern associated with the defective β-cell shows a reduced first phase (which reproduces the response of a subject in the initial stage of type 2 diabetes). The insulin-secretory response to the meal-like study (Fig. 10, middle right) shows only a modest sign of biphasicity, i.e., a little bump at 20 min followed by a subsequent surge. The corresponding profile of peripheral insulin concentration increases without any clear sign of biphasicity (Fig. 10, bottom right). In the simulation of the defective β-cell, no sign of biphasicity is present in either insulin secretion or peripheral insulin concentration. Compared with the defective β-cell, the normal β-cell determines a more rapidly increasing early phase. In fact, at 20 min, the normal insulin profile has already achieved 71% of the maximal insulin level attained at 60 min, whereas the defective insulin profile is only at 33% (Fig. 10, bottom right). All in all, it appears that a healthy β-cell, showing a biphasic response to a step increase in glucose concentration, generates an early insulin response to a meal-like study that rapidly elevates portal and peripheral insulin concentrations. Conversely, a defective β-cell, showing an impaired first-phase insulin

Fig. 10. Results of the model simulation aimed to gain insights into the relationship between the insulin response to a brisk intravenous glucose challenge and the insulin response to a gradual elevation of glucose following food ingestion. The model of Toffolo et al. (113) was employed to predict β-cell secretion and the corresponding profile of peripheral insulin concentration in response either to a square wave of hyperglycemia or to a gradual glucose increase mimicking a meal. The model operated under conditions reproducing either a normally functioning or a defective β-cell. Top: glucose profiles reproducing either a square wave of hyperinsulinemia (left) or a meal-like study (right). Middle: actual average glucose concentrations measured during a meal in normal humans (5). Middle: corresponding model predictions of incremental (above basal) insulin secretion under the assumptions of a normal or a defective β-cell. Bottom: corresponding peripheral insulin concentration profiles. Interpretation of the results is given in the text.
response to a step increase in glucose concentration, generates a sluggish response to a meal-like study.

There is experimental evidence supporting the conjecture that the first-phase insulin response to a rapid intravenous glucose challenge and the early insulin response to oral glucose are emanations of common underlying β-cell mechanisms. For instance, the AIRGlc has been shown to be correlated, albeit not strongly, with the insulinogenic index measured 30 min after glucose ingestion (the correlation coefficient was 0.61 in Ref. 84 and 0.54 in Ref. 54). The correlation between insulin secretion indexes derived from an IVGTT and a meal has recently been confirmed with the minimal-model approach in a study comparing glucose tolerance in young and elderly subjects (4). Also, the first-phase insulin response to the hyperglycemic clamp and a model-based index of β-cell function during the OGTT have been shown to be correlated (11). Another evidence that the insulin responses to intravenous and oral glucose are closely related comes from the observation that, in patients with IGT or in the early stages of type 2 diabetes, the first-phase insulin response to an IVGTT or a hyperglycemic clamp is almost invariably impaired, and this defect is mirrored by a more sluggish early insulin response following glucose ingestion (28, 101) (the role of phasic insulin release in clinical diabetes is reviewed in Refs. 32, 35, 97, and 120).

So far, we have dealt with insulin secretion under physiological conditions, focusing on the role of glucose only. This has been done because glucose is the most important factor affecting insulin secretion. However, it is well known that intravenous glucose elicits a smaller insulin response than oral glucose (42, 53, 83). In this regard, it can be noted that the incremental insulin levels achieved in our simulation of a meal-like study (i.e., a study in which glucose is infused intravenously in such a way as to mimic the postprandial plasma glucose profile; see Fig. 10) are approximately one-half of those normally achieved during a real meal (175 vs. 350 pmol/l). The increment in insulin response observed after oral compared with intravenous glucose administration is interpreted as the presence of other factors besides glucose that contribute to stimulate β-cell secretion, such as the neurally activated cephalic phase and gastrointestinal hormones (incretins). The cephalic phase is a short-lived β-cell secretion that is evoked by the sight, taste, and smell of food (see Refs. 2 and 63 and references therein). It precedes food ingestion and is mediated by pancreatic innervation. The cephalic phase causes a rapid (≤2 min) but small (≈30 pmol/l) increase in plasma insulin concentration. Albeit modest in absolute terms, the cephalic phase plays a significant physiological role, as an inverse relationship between the cephalic phase and the initial increase in plasma glucose concentration has been found (17). Incretins are gastrointestinal hormones that have a potentiating effect on β-cell secretion after ingestion of a carbohydrate meal (15, 38, 117). It has been suggested that the incretin effect is responsible for ∼50% of the insulin response to oral glucose. It follows that incretin deficiency may contribute to the development of postprandial hyperglycemia and that administration of exogenous incretin hormones may have therapeutic applications in the treatment of type 2 diabetes. The presence of factors that contribute only to the insulin response to ingested glucose, but not to the insulin response to intravenous glucose, may explain why the correlation between the AIRGlc and intravenous glucose and the insulinogenic index measured 30 min after glucose ingestion is not strong.

**EFFECTS OF EARLY INSULIN RESPONSE ON PRANDIAL GLUCOSE METABOLISM**

An increasing body of evidence indicates that the early insulin response following glucose ingestion plays a critical role in the maintenance of postprandial glucose homeostasis. The early surge in insulin concentration is capable of limiting the initial glucose excursion mainly through the prompt inhibition of endogenous glucose production, with the insulin-mediated curtailment of glucagon secretion being particularly relevant (75, 76, 102). This confirms, under physiological conditions, the findings of the studies that assessed the metabolic relevance of the first-phase insulin response to intravenous glucose. In the following discussion, we will provide a brief synopsis of the studies that assessed the benefits associated with the early phase of insulin release as well as the problems that are associated with its impairment.

We previously mentioned the study by Calles-Escandon and Robbins (18) in relation to the physiological relevance of the first-phase insulin response to intravenous glucose. In that study, Calles-Escandon and Robbins also studied the effects of selective suppression of the early insulin response (0–30 min) to oral glucose by using a brief intravenous infusion of somatostatin. They found that the loss of the early phase during the OGTT was associated with reduced glucose tolerance. In fact, a transient hyperglycemia that stimulated a sustained late insulin response between 60 and 120 min was observed.

Further experimental evidence that the early insulin response is associated with glucose tolerance comes from a study conducted on IGT patients by Mitra Kou et al. (75). That study revealed an inverse correlation between the plasma insulin level achieved 30 min after an oral glucose load and the plasma glucose concentration measured at 120 min. This confirms that a defective early insulin response may lead to postprandial hyperglycemia, which in turn determines a prolonged stimulation of the β-cell leading to hyperinsulinemia.

Basu et al. (3) carried out a study to understand the role of the pattern of insulin delivery on the ability to dispose a glucose load. They studied nondiabetic, obese, and diabetic individuals during a meal-like study in which somatostatin was used to inhibit endogenous insulin secretion while glucose was infused in such a way as to mimic the rate of entry of glucose from the gut following the ingestion of 50 g of glucose. During the study, the same amount of exogenous insulin was infused in such a way as to mimic the postprandial insulin profile of either a normal or a diabetic individual, the latter profile featuring a sluggish pattern of insulin delivery (corresponding to a loss of early insulin secretion). It was found that the “diabetic” insulin profile was associated with a higher glucose peak and a prolonged duration of hyperglycemia in all groups (Fig. 11).

Kahn et al. (61) have shown that the relationship between the early insulin response and glucose tolerance is hyperbolic in nature. This result is reminiscent of the hyperbolic relationship between the insulin response to intravenous glucose and insulin sensitivity that was originally suggested by Bergman et al. (8) and then investigated thoroughly by Kahn et al. (60). One important consequence of this nonlinearity is that even
small decreases in the early response to glucose ingestion can have dramatic effects on the postprandial glycemic profile in subjects who are glucose intolerant.

A reduced early insulin response to glucose ingestion is quite a common defect in type 2 diabetes, and this abnormality can be a contributing factor in the development and progression of the disease. In fact, in patients with impaired glucose tolerance or in the early stages of type 2 diabetes, the impairment of the early insulin response contributes to postprandial hyperglycemia. This maintains the β-cell under a condition of prolonged stimulation, which eventually leads to late-phase hyperinsulinemia. Chronic postprandial hyperglycemia and hyperinsulinemia have detrimental effects on the glucose-insulin system. Chronic hyperglycemia is capable, through the mechanism of glucotoxicity (99), of further worsening β-cell secretion. Chronic hyperinsulinemia may lead to β-cell exhaustion, may cause downregulation of the insulin receptor thus increasing insulin resistance (32), and may produce the well-known negative consequences on the vascular endothelium (106, 111). This vicious cycle plays a significant role in the pathogenesis and evolution of type 2 diabetes [as thoroughly examined in some recent surveys (34, 59, 97)]. Thus interventions to restore the early insulin surge should contribute to improving glucose tolerance in type 2 diabetic patients. In a study by Bruce et al. (16), type 2 diabetic patients received an identical dose of insulin in three different regimens at mealtimes: insulin administered intravenously over 30 min at the beginning of a meal in such a way as to mimic a normal early insulin response, with the same profile but delayed by 30 min, and as a constant infusion over 60 min. The best result in terms of postprandial glucose tolerance was obtained with the early administration of insulin, in keeping with the proposed direct effect of the early insulin response on hepatic glucose production. The beneficial effect of a short intravenous insulin infusion mimicking the early insulin response in type 2 diabetic patients has been subsequently confirmed by Luzio et al. (69).

Because intravenous infusions aimed at achieving an early and adequate supply of insulin are clearly impractical, simpler alternatives, applicable to everyday life, have been devised to correct a defective early insulin response in diabetic patients whose disease is inadequately controlled by diet and exercise. One is the subcutaneous administration of fast-acting insulin analogs; another is the use of pharmacological agents (such as repaglinide, nateglinide, and similar compounds) that exert a preferential stimulation of the early insulin secretion in those patients still having a residual β-cell function. Such therapeutic approaches have been shown to be effective in reducing postprandial glucose and insulin excursions in type 2 diabetic patients, as illustrated in recent review papers by Del Prato and colleagues (34, 36).

FROM BIPHASICITY TO PULSATILITY

In the previous sections, we argued that the key feature of insulin secretion under physiological conditions is not the biphasic shape but rather the ability to rapidly elevate insulin levels in the bloodstream. This interpretation is further strengthened by recently obtained insights into the oscillatory nature of insulin secretion following physiological stimuli. When the β-cell response during a meal is being assessed, insulin and C-peptide are usually sampled in the peripheral blood during a meal-like study in which endogenous insulin secretion was inhibited with somatostatin and glucose was infused so as to mimic the rate of glucose entry from the gut during a meal. The same amount of exogenous insulin was infused mimicking either the normal postprandial insulin profile or the one typically found in type 2 diabetic patients, in which the early insulin response is impaired. The latter insulin profile was associated in all groups with a more elevated glucose peak and a more prolonged hyperglycemia. Data are replotted from Basu et al. (3) with the permission of the Journal of Clinical Investigation.
when deconvolution is used to reconstruct insulin secretion, as done by Porsken and coworkers in a series of publications [a comprehensive summary of which is provided by the same group of researchers in two recent review papers (89, 91)]. These investigators have shown that insulin secretion during a meal has an oscillatory nature and consists of a sequence of pulses whose amplitude and frequency are modulated by glucose (the frequency of secretory bursts is ~6 min per pulse) (90). Also, the incretin effect appears to be mediated via the pulsatile mode of secretion (92). Thus, even though C-peptide (90) with the permission of the ©

Even though the early secretory pattern during a meal is not as smooth as that predicted by the β-cell model simulation (Fig. 10), the crucial point nevertheless remains the ability of the β-cell to generate an insulin response that rapidly exposes the liver to high insulin levels. The fact that such high insulin levels have an up-and-down pattern is a bonus that renders insulin action on the liver more effective. In fact, many studies have shown that hepatic insulin action is improved when insulin is presented to the liver in an oscillatory fashion (as reviewed in Refs. 73, 82, and 91). Interestingly, type 2 diabetic patients show profound defects in all aspects of pulsatile insulin release. Alterations of insulin pulsatility in the postabsorptive state have been identified as a very early sign of β-cell dysfunction in IGT and type 2 diabetic patients (74). After glucose ingestion, pulses occur less regularly and have significantly lower amplitude in type 2 diabetic patients (91). Given the considerable importance of the pulsatile manner of insulin secretion in optimizing insulin effects on target tissues, restoration of the physiological pulsatility of early insulin secretion might be a valuable therapeutic approach in the management of type 2 diabetes and influence the future tailoring of antidiabetic drugs (100). In this respect, it is encouraging that recent data obtained in healthy subjects have shown that the insulinotropic agent repaglinide, which is used to stimulate early insulin secretion in type 2 diabetic patients, amplifies the insulin burst mass while preserving insulin pulsatility (58).

**SUMMARY**

Insulin secretion is not intrinsically biphasic. Insulin secretion can occur in a biphasic manner depending on the type and magnitude of the glucose stimulus. The biphasic shape is a feature that insulin secretion exhibits when the β-cell is exposed to a rapidly changing glucose concentration. Under physiological conditions, when glucose increases gradually, the first and second phases are not clearly distinguishable.

The same dynamic features of the β-cell machinery that determine a pronounced first-phase insulin secretion in response to a rapid intravenous glucose administration also ensure a prompt release of insulin into the bloodstream in response to a meal. Conversely, the same defect that determines a marked reduction in first-phase insulin secretion induces a sluggish increase of insulin in response to a meal. Therefore, even though the ICGTT or the hyperglycemic clamp determine conditions that do not exist in nature, they are valuable tools that allow investigators to pinpoint defects that are likely to have an impact on the secretory response during normal physiological situations.

Both animal and human studies indicate that the first-phase insulin response to intravenous glucose has beneficial effects on the regulation of glucose metabolism. In particular, the first phase has a profound and long-term inhibitory effect on hepatic glucose production. Likewise, the early insulin response to ingested glucose is an important determinant of prandial glucose tolerance. The impairment of early insulin release commonly found in diabetic patients determines a prolonged duration of postprandial hyperglycemia and, in turn, a compensatory late-phase hyperinsulinemia. Therapeutic interventions capable of restoring the early insulin release ameliorate glucose tolerance in such patients.

Recent studies using frequent portal venous sampling and deconvolution indicate that insulin secretion following glucose ingestion increases in an oscillatory fashion. Thus insulin secretion in real life is pulsatile rather than biphasic. Although such insulin pulsatility is considerably dampened at the systemic level, it is likely to exert a priming effect on the liver.

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**Fig. 12.** Pulsatile insulin secretion before and during a meal assessed by intraportal sampling. Portal vein insulin concentrations in the basal state (**left**) and after meal ingestion (**right**). The insulin profiles to which the liver is exposed in response to a meal are dynamic and resemble an on/off pattern. Reproduced from Porsken et al. (90) with the permission of the American Journal of Physiology.

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This further underlines the notion that insulin shape and function are closely related.

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