Journal of Cellular Biochemistry 48:3–11 (1992)

PROSPECTS

Desensitization of the Insulin-Secreting Beta Cell

Gerold M. Grodsky and Janice L. Bolaffi

Metabolic Research Unit, University of California, San Francisco, California 94143

Abstract In human diabetes, inherent impaired insulin secretion can be exacerbated by desensitization of the β cell by chronic hyperglycemia. Interest in this phenomenon has generated extensive studies in genetic or experimentally induced diabetes in animals and in fully in vitro systems, with often conflicting results. In general, although chronic glucose causes decreased β-cell response to this carbohydrate, basal response and response to alternate stimulating agents are enhanced. Glucose-stimulated insulin synthesis can be increased or decreased depending on the system studied. Using a two-compartment β-cell model of phasic insulin secretion, a unifying hypothesis is described which can explain some of the apparent conflicting data. This hypothesis suggests that glucose-desensitization is caused by an impairment in stimulation of a hypothetical potentiator singularly responsible for: 1) some of the characteristic phases of insulin secretion; 2) basal release; 3) potentiation of non-glucose stimulators; and 4) apparent "recovery" from desensitization. Review of some of the pathways that regulate insulin secretion suggest that phosphoinositol metabolism and protein kinase-C production are regulated similarly to the theoretical potentiator and their impairment is a major contributor to glucose desensitization in the β cell.

Key words: insulin secretion, desensitization, insulin secretion model, secretion kinetics, protein kinase-C

Impaired β-cell secretion in the face of chronic hyperglycemia is characteristic of non-insulin-dependent, and early stages of insulin-dependent, diabetes [1]. Reduction of the blood sugar with insulin, sulfonylureas, or diet improves insulin release and glucose homeostasis [2], indicating that glucose-induced desensitization is a contributory factor in addition to the underlining β-cell lesion or insulin resistance characteristic of these disease states [1]. Desensitization of the β cell also occurs with continuous stimulation by other secretagogues, but given the importance of glucose as a key physiological regulator of β-cell secretion, many studies have focused on β-cell desensitization to this fuel carbohydrate [3].

For the most part, β-cell function has been investigated in the isolated perfused pancreas or islets from animals with hyperglycemia caused by genetic or experimental lesions in the pancreas, or in freshly isolated normal islets continuously stimulated with glucose [4,5–9]. The characteristics of β-cell desensitization derived from these experimental studies is the subject of this review.

Received September 4, 1991; accepted September 4, 1991.

Address reprint requests to Gerold M. Grodsky, Metabolic Research Unit, University of California, HSW 1157, San Francisco, CA 94143.

DESENSITIZATION TO GLUCOSE IN VIVO

Genetic models used to study hyperglycemic animals include the obese SHR-N-cp [8] and the Zucker fa/fa [10] rats. The most common models with experimentally induced hyperglycemia are adult mildly hyperglycemic rats, given a low dose of streptozotocin neonatally, or partially pancreatectomized rats [9,11,12]. Because of inherent complexities, it is not always possible to distinguish effects of desensitization by hyperglycemia per se from the inherent β-cell lesions in the model [13]. Nevertheless, these models are still useful since genetic, environmental, and hyperglycemic effects are variable contributors to human diabetes. As a further complexity, isolated islets from these animals are required for most metabolic studies rather than the intact pancreas, which is 99% acinar tissue. Since impaired response to glucose in islets from hyperglycemic animals can be partially reversed by surprisingly brief periods of low glucose (1–2 h) [8,12,14], results with islets are highly dependent on the ambient glucose and possibly other factors in media used during the isolation procedure [15].

Notwithstanding the different models investigated, some β-cell functional changes in these chronic hyperglycemic states are consistently observed [8,9,11,16,17]. Insulin secretion, in re-

sponse to glucose, either as a direct stimulus or as a time-dependent priming agent, is severely reduced or absent. Paradoxically, there is an increased basal release in low glucose and an exaggerated response to alternate secretagogues known to require glucose as a potentiator of their action (e.g., arginine, carbachol, α-ketoisocaproic acid). Thus, while a specific defect in glucose recognition occurs during glucose-induced desensitization, the potentiating features of glucose action are retained or enhanced.

Glucose normally increases insulinogenesis both at the translational (minutes) and transcriptional (hours) levels. Though results are conflicting, insulinogenesis and insulin m-RNA production are usually impaired in genetic or experimentally induced hyperglycemic animal models [9,18,19].

Reversal of desensitization in these animal models requires days of experimentally induced euglycemia [9,11,20]. This may reflect the important clinical observation in humans that a period of intense glucose normalization for several days can improve β-cell function for extended periods [1,2]. In sharp contrast is the observation that glucose sensitivity of the isolated pancreas from these models can be partially recovered in only an hour of low glucose in vitro [12,14]. As noted later, however, this in vitro "recovery" could be a measure of the residual time-dependent potentiation caused by glucose, rather than an improvement in glucose-regulated terminal secretion.

As an alternate to the use of hyperglycemic animals with induced or genetic β-cell defects, investigators have studied desensitization of insulin secretion from normal, ambulatory rats made hyperglycemic by continuous infusion of glucose [14,15,21]. This model permits evaluation of the effects of glucose per se, on normal β cells. (Although the actual impact is unknown, it is recognized that the amount of glucose infused is calorically sufficient to interfere with normal food consumption which in turn could indirectly affect results.) In these models, hyperglycemia again results in islet or pancreas desensitization to glucose, increased basal secretion, and potentiation of alternate secretagogues. Onset of these effects is not immediate, but requires 24–48 h of continuous hyperglycemia [21]. In contrast to the other animal models, chronic glucose in normal rats causes increased insulinogenesis [22,23]. This observation suggests that impaired insulinogenesis, when observed, is characteristic of underlying β-cell lesions and not the hyperglycemia [19]. Thus, as in fully in vitro preparations (discussed later), glucose-induced desensitization of the β cell is at the level of insulin release, not synthesis.

DESENSITIZATION IN VITRO

Phasic Insulin Secretion and Synthesis

In fully in vitro systems using perifused or batch-incubated islets [4], the insulin secretory response to constant glucose stimulation is characterized by a brief, first-phase release followed by a progressively increasing second phase, generally peaking at 1.5–3 h (Fig. 1). This gradually increasing phase of secretion reflects the ability of glucose (and other secretagogues) to amplify its own signal and is referred to as time-dependent potentiation or priming [3,24,25,26]. Third-phase, or desensitized, secretion ensues with a spontaneous decline to low levels which are sustained at 15–25% peak secretion for at least 48 h [27]. Since secretion from islets may reflect artifacts of preparation, it is important to note that the third phase of insulin secretion is also seen in the isolated perfused pancreas [5,28]. In contrast to the observations with hyperglycemic animals, onset of desensitization (third phase) in fully in vitro systems is relatively rapid (Fig. 1). However, recovery of secretory function after reducing glucose is long, requiring more than 3 h (unpublished observations) and may be similar to the hours of normalization required to reverse desensitization in the intact diabetic animal and the human diabetic [reviewed in 1]. Some reduction of secretion from isolated islets can be attributed solely to time in culture resulting in diminution of the total secretory response [4,5,29], but the phasic patterns are retained [3].

Over 24 h of chronic glucose there is little change in the rate of insulin synthesis measured by ^{3}H-leucine incorporation [30]. Similarly, glucose-regulated proinsulin to insulin conversion does not decline during third-phase secretion [30]. Thus, glucose-induced desensitization is specific to the secretory mechanism, and other glucose-regulated steps in insulin production in the β cell are not desensitized. Since synthesis is maintained, or increased with time, and enzymes regulating glucose metabolism (e.g., glucokinase) can be induced [31], extended culture of islets for days or weeks can result in improved release of insulin and compensation for desensitization [11,32,33]. Similar improvement per-

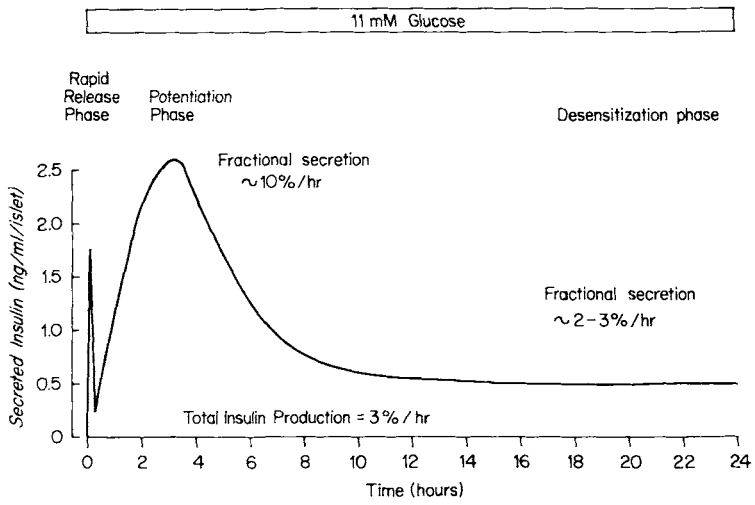

Fig. 1. Schematic of the characteristic three phases of insulin secretion during constant stimulation of islets with glucose (data from [27]).

haps would not be expected in animals or humans with an additional genetic or experimentally induced β-cell lesion.

It is unlikely that, in fully in vitro conditions, desensitization of secretion can be attributed to depletion of total islet content; depending on the glucose concentration, the magnitude of the secretory decline to third phase is 2–3 times the decline in total islet insulin content ([4,27] and Fig. 1). Islets stimulated with different glucose concentrations show similar temporal secretory patterns of stimulation-desensitization differing only in total amounts of insulin secreted [3]. Thus, depletion of a single finite compartment is not indicated. A possible interpretation of such secretory patterns which cannot as yet be excluded is that third-phase release reflects depletion of threshold-sensitive β cells which are recruited in increasing numbers by increasing glucose concentrations. Glucose-dependent recruitment of heterogeneous β-cell populations has been theoretically modeled [24] and elegantly demonstrated [34]. Nevertheless, the most likely possibility is that desensitization is caused by down regulation of signal-related functions controlling insulin release. Since many non-glucose secretagogues cause third-phase insulin release, it is probable that the function involved is inherent in the basic secretory process.

Pulsatile Stimulation

In a limited study, presentation of glucose in vitro as pulses has not prevented third-phase secretion [4]. However, the potential role of oscillatory stimulation to prevent desensitization cannot be discounted given the extreme sensitivity of secretory systems to specific changes in amplitude and frequency of the secretory signal, and particularly since endogenous insulin secretion oscillates at a much higher frequency than the pattern tested [35].

Role of Paracrine Hormones

It is unlikely that in the intact pancreas, glucagon or somatostatin from peripheral α or δ cells reach the core β cells since islet afferent circulation, in situ, flows from the core to the periphery [36]. Thus, although paracrine effects on third-phase insulin secretion are probably of little import in vivo, such a role should be considered in isolated islet preparations where glucagon or somatostatin can reach the β cell by simple diffusion. With continuous glucose, somatostatin secretion declines, not increases, indicating that low third-phase insulin secretion (Fig. 1) is not caused by an inhibition coincident with elevated somatostatin [37]. Additionally, β cells show no increased sensitivity to somatostatin since responses to acute somatostatin challenges are similar in fresh or desensitized islets. Though glucagon secretion also declines during chronic glucose, which can account for the diminished third-phase insulin secretion, coinfusion of excess glucagon is not preventative nor is a decreased sensitivity of β cells to glucagon demonstrable [37]. The recent report that chronic glucose produces third-phase desensitization of

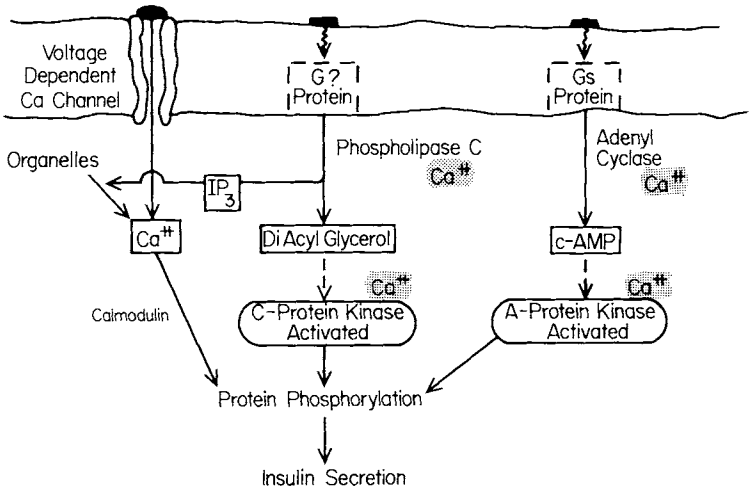

Fig. 2. Major pathways by which glucose or modulators regulate insulin secretion. Stimulators can provide cytosolic Ca^{++} by increasing extracellular uptake or by mobilizing organelle stores. Ca, in turn, positively regulates the pathways at various sites as indicated, Ca^{++}.

insulin secretion in a pure β-cell preparation [7] further suggests that desensitization is not mediated by paracrine hormones. Though peripheral to this review, the decline in glucagon or somatostatin secretion during their chronic stimulation emphasizes that desensitization phenomena will become a consideration also for the regulation of the non-β cells in islets.

MODEL ANALYSIS OF STIMULATION—DESENSITIZATION

The metabolic functions controlling insulin secretion are both complex and interrelating (Fig. 2). Since it is clearly impractical for any laboratory to simultaneously monitor all of the involved pathways and their interrelationships, clarification of the kinetic characteristics required for a modulator of insulin secretion may help establish which pathway or intermediate predominates in a given metabolic circumstance.

Previously, a two-compartmental mathematical model was developed which quantitatively duplicates first- and second-phase insulin release when tested in the perfused pancreas by a variety of stimulating conditions and kinetic patterns of glucose presentation [24,38]. A schematic of this model is shown in Figure 3 which describes the different phases of insulin secretion illustrated in Figure 1. The compartments are shown as containing insulin although they could also represent a secretion signal used up in the secretion process. First-phase release is the result of a rapid emptying of the small compartment of stored insulin and is complete within 5–10 min of the initial stimulation. The second phase is driven by a hypothetical potentiating agent, P, which accumulates during constant stimulation and thereby produces a proportional increase in secretion during the first 2 to 3 h. An important feature of the potentiator P is that it is not used up during secretion but is degraded independently of glucose concentration with a half-life of 20 to 60 min. When glucose stimulation is stopped both insulin release and production of P is arrested. However, P, though decreasing with time, continues to cause accumulation of insulin in the small compartment. Thus, residual P produces a memory factor in the β cell; after a brief rest, restimulation can produce a hyperinsulin release. This memory function has long been recognized as an important action of glucose in the β cell and is referred to as time-dependent potentiation or priming [25,38].

Although experiments appear similar amongst laboratories, there is disagreement as to whether chronic glucose for 1 to 2 days actually causes desensitization of insulin secretion from the β cell in fully in vitro islet preparations [6,7,13,15]. Usually fresh islets are stimulated for test periods with glucose or alternate secretagogues for 30 min to 2 h, then cultured with high or low glucose. Afterwards the islets are washed and retested with the original test stimulation. When taken in context of the compartmental model, conclusions based on such "before" and "after" tests can be highly dependent on the experimen-

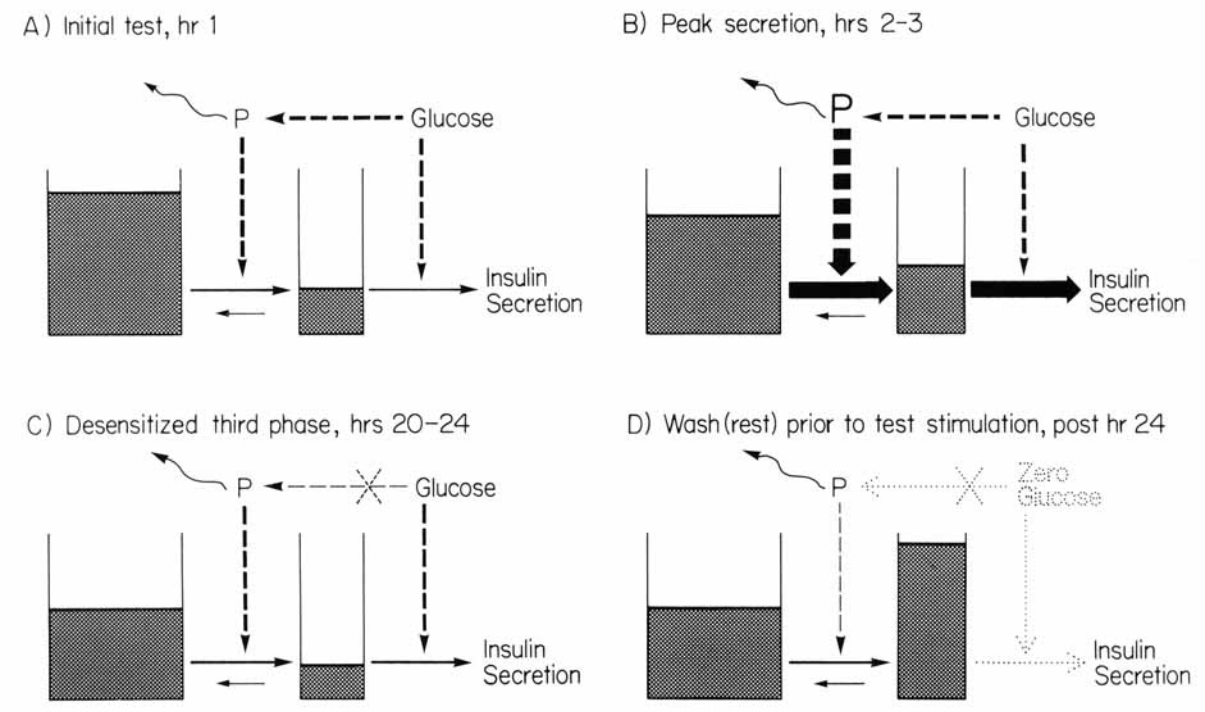

Fig. 3. Schematic representations of a two compartmental model of insulin secretion [for mathematical development see 24,38]. Model assumes glucose stimulates insulin secretion from the small labile compartment and also increases a potentiating signal, P, which provides additional insulin from the large compartment. **A:** At 1 h, the small compartment has partially emptied (first phase secretion) and P is increasing (second-phase secretion). **B:** At 2–3 h, P and second-phase secretion is maximized. **C:** At 20–24 h, postulated defect in production of P has resulted in third-phase insulin secretion (desensitization) as indicated by X. **D:** During a rest period at low glucose, insulin secretion and production of P stops but residual P continues to provide insulin to the small compartment, resulting for a time in overfilling. On restimulation, a hyperresponse of particularly first-phase secretion occurs (not shown).

tal design. Thus, the length of the test periods can be critical—with longer periods, total insulin release is a greater reflection of the normally increasing potentiator P. The initial test period is also dependent on the stabilization time used after the rigorous techniques required for isolation. Most importantly, when islets are rested (washed at low glucose) and retested after chronic glucose, results are exquisitely sensitive to the usually arbitrary rest periods selected. Figure 4 shows that islets cultured with glucose and stimulated after a 30-min rest produce a characteristic hyperinsulin response greater than that of the initial test stimulation at h 1. When rest periods are extended, secretion progressively declines to that seen previously at h 20–22. This level may be equivalent to (as shown in Fig. 4) or even lower [4] than for the original stimulus. Thus, depending on the rest periods chosen, glucose test restimulations can cause more (potentiation) or less (desensitization) insulin release than the initial test stimulus. These declining responses with increasing length of rest periods correspond in the model to the residual potentiating factor P and its slow degradation with time during a wash in the absence of glucose. We emphasize that these wash-retest experiments actually evaluate residual time-dependent potentiation, not desensitization. A similar residual potentiation can explain the hyperresponse to other secretagogues (e.g., arginine or α-ketoisocaproic acid) [4,6,9] when tested after chronic glucose. It also may explain the improved response of the perfused pancreas to glucose when rapidly removed from hyperglycemic animals and stimulated after 30–60 min of low glucose, an observation usually interpreted as reversal of desensitization [12,14]. With islets exposed to chronic low glucose, P is theoretically not elevated and restimulation test responses are always comparatively small and less dependent on the rest periods employed (Figs. 3, 4).

Basal release during the rest periods after chronic elevated glucose is high ([7,15] and Fig. 4). From the model it is our contention that basal release is also driven by the residual poten-

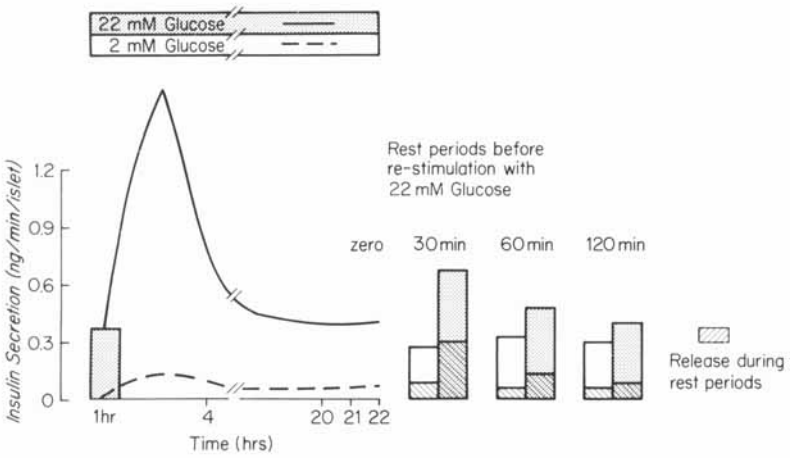

Fig. 4. Effect of test stimulations on isolated islets before and after chronic glucose. Lines are insulin release during chronic high (22 mM, ——) and low (2 mM, – – –) glucose. Bars are insulin release during 1 h tests with 22 mM glucose before and at different rest periods after chronic stimulation [from 64].

tiation and that release is being provided by minor insulin secretagogues in the culture media.

Regardless of the time periods employed, if the restimulation response is compared to peak secretion at h 2–3 (Fig. 4), it is obvious that insulin secretion, in response to continuous glucose, is severely reduced during the third phase of insulin secretion. Model analysis suggests that *this decline is caused by a decreased ability of glucose to produce potentiator.* Therefore, practically all the characteristics of desensitization studied in vitro or with animal models, including time-dependent potentiation, desensitization, high basals, and declining response to test stimuli with increasing rest periods, are modulated by the hypothetical potentiator, P. This hypothesis can be tested further by examining minute-to-minute phasic insulin release during the wash-retest stimulation after chronic glucose. From the model, it would be predicted that the superfilled first compartment would result in an enhanced first phase, whereas the inability for glucose, in the desensitized islets, to produce P normally, would result in a poor second phase. That this is the case has been recently reported [12,39].

POSSIBLE METABOLIC SITES OF DESENSITIZATION

Multiple metabolic events contribute to the stimulus-secretion coupled cascade and are potential sites for regulation and desensitization of secretion (Fig. 2). Some of these include: 1) glucose transport and metabolism; 2) intracellular Ca generated by plasma membrane Ca flux or mobilization from intracellular stores; 3) cAMP production; and 4) membrane phospholipid hydrolysis with subsequent elucidation of diacylglycerols, inositol polyphosphates, phosphatidic acid, and arachadonic acid.

Glucose Transport and Metabolism

Glucose transport into the normal β cell, although the obligate first step in glucose metabolism required for secretion, is normally not rate limiting; decrements to 1–10% of normal are probably required to effectively reduce glucose availability. Nevertheless, changes in the glucose uptake machinery of the β cell may occur during extended exposure to high glucose and contribute to observed desensitization of insulin secretion. In several diabetic animal models such as the BB or the Zucker fa/fa rats, the onset of diabetes is marked by decreased glucose uptake by the β cell as well as decreased expression of mRNA for the β cell specific glucose transporter II [reviewed in 10]. However, glucose uptake is not decreased in pancreas from rats made hyperglycemic by neonatal streptozotocin treatment [40,41] or by glucose infusion for 2–7 days [42]. In normal isolated rat islets whose secretion is desensitized by chronic glucose in vitro, 3-O methyl glucose uptake is not changed from fresh islets or saturated at 22 mM glucose (Fig. 5). Thus, impaired glucose transport may contribute to diminished secretory states in some instances of genetic diabetes but does not appear to be a consistent factor in glucose-induced desensitization or related to the putative potentia-

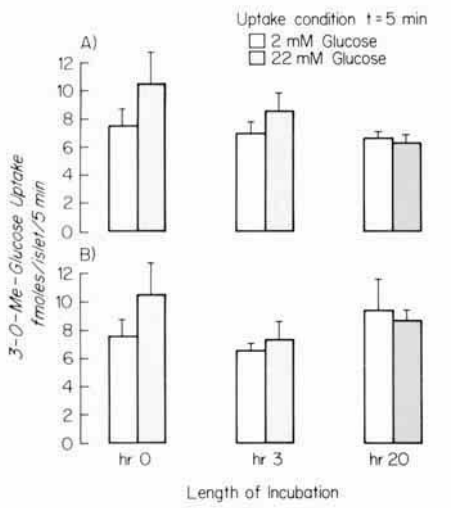

Fig. 5. Comparison of ^{3}H 3-O-methyl glucose uptake in islets chronically incubated for 0, 3, or 20 h with glucose. (A = 2 mM; B = 22 mM). Times correspond to early, maximal, and desensitized insulin secretion (Fig. 1). Uptake was measured after 5 min of incubation in Krebs-Ringer bicarbonate buffer with ^{3}H 3-O-methyl glucose in the presence of 2 (□) or 22 (▨) mM glucose. [For technical details, see 65.]

tor, P. The possibility that a defect in mitochondrial oxidation of glucose is involved in desensitization has been suggested [41].

Calcium

Changes in Ca uptake and intracellular free Ca are considered to be key in β-cell signal transduction at several metabolic levels ([43,44] and Fig. 2) and could be involved in glucose-induced desensitization. Unfortunately, measurements of free Ca with sensitive techniques using Fura or Indo dyes are restricted to studies with dispersed β-cell preparations which may not retain normal desensitization features.

Direct measurement of Ca uptake in islets has shown little change after 3 h of glucose, when secretory rates are near maximum [45], or after 24 h when insulin secretion is desensitized [46,47]. Although Purrello et al. find that K-channel flux (measured by Rb efflux) is enhanced in glucose-desensitized islets [48], an effect which should inhibit voltage-dependent Ca channels, Ca currents are actually maintained [49]. Furthermore, in islets already desensitized to glucose, voltage-dependent Ca channels are still sensitive to acute inhibition by channel blockers such as verapamil [45]. Thus, gross changes in Ca-channel activity are probably not the cause of glucose-induced desensitization. However, other aspects of Ca metabolism, including changes in intracellular distribution or decreased cellular Ca sensitivity, should still be considered [50].

Because of its ubiquitous role in β-cell metabolism, Ca is probably involved in the steps regulating the potentiator (Fig. 3), but its identification as the potentiator itself is less likely.

cAMP

Since cAMP is such an important potentiator of insulin secretion (Fig. 2), its role in glucose-induced desensitization has been evaluated. Addition of IBMX or forskolin chronically with glucose does not prevent or delay third-phase release from normal islets and sensitivity to these agents is similar in fresh or desensitized islets (4). Furthermore, response to these cAMP enhancers is usually not impaired in the perfused pancreas from hyperglycemic animal models [12,17]. Thus, a major role for this second messenger in mediating third-phase secretion is not likely. Glycogen levels in β cells with hyperglycemia are increased and the possibility that glucose metabolism during desensitization is diverted to synthesis of glycogen which can be mobilized for potentiation has been suggested [14].

Protein Kinase C

The role of protein-kinase-C (PKC) in glucose-stimulated insulin secretion has been the subject of intense recent investigation and of some controversy [51–53]. However, evidence is strong that induction of membrane phosphatidylinositol (PI) hydrolysis, which mediates 1,4,5 inositol trisphosphate (IP_3) and diacylglycerol (DAG) production, and the subsequent activation of PKC, is important for amplifying insulin secretion. The disagreement lies in whether glucose itself is sufficient to initiate PI hydrolysis and activation of PKC, or if additional potentiators such as carbachol or cholecystokinin are required. Part of the controversy may arise from the comparison of data obtained with fresh islets opposed to those cultured overnight with glucose and possibly already desensitized. It is reported that in freshly isolated islets, glucose stimulates PI turnover [54] and PKC translocation to the plasma membrane [55], and that added carbachol stimulates this reaction further. In islets cultured overnight (desensitized?), the addition of potentiators with glucose appears to be necessary to

detect PI hydrolysis, generation of DAG, or PKC activation [51,56,57].

There is further substantial evidence suggesting that the PKC pathway is an important potentiator of the rising, second phase of insulin secretion and that loss of ability of glucose to activate this pathway may contribute to glucose-desensitized secretion. PI turnover diminishes concomitant with third-phase insulin secretion in islets continuously stimulated with glucose [58]. In the presence of added carbachol, the decline of second-phase secretion may be associated with a depletion of endogenous enzyme [59–61]. In third phase, insulin secretion loses responsiveness to stimulation of PKC by phorbol ester and to inhibition by staurosporine [45,62]. Thus the *ability* to activate PKC is suppressed in glucose-desensitized islets. This may reflect changes in intracellular levels of Ca or other regulatory elements, particularly DAG or its precursor phosphatidic acid, both generated from glucose-derived trioses [57,63]. The complexity of the PI pathway is underscored by the observation that PI hydrolysis is still capable of stimulation by carbachol sufficient to elicit unimpaired insulin responses in glucose desensitized islets [45]. Regardless, a product(s) of the phosphoinositol pathway (DAG?, IP_3?) is an attractive candidate to be the potentiator of second-phase insulin release and the site of desensitization (Fig. 3).

GENERAL DESENSITIZATION TO STIMULATORY AND INHIBITORY AGENTS

In this review we have evaluated several aspects of glucose-induced desensitization and propose a model to explain current information on this subject. However, a last interesting aspect of β-cell secretion is of note. Glucose-stimulated insulin secretion of islets not only desensitizes to continuous perifusion with stimulatory agents such as glucagon or carbachol [37], but to inhibitory agents as well. Yet these agents are fully active if added acutely at a time when their chronic effect would have waned. This is illustrated in Figure 6 [45] where the inhibitory effect of verapamil (presumably on the voltage-dependent Ca channel) is lost with continuous, but not acute, administration. Similarly, the inhibitory effect of somatostatin diminishes during chronic exposure while inhibition by an acute challenge is retained [37]. This phenomenon is not universally observed since inhibition to trifluoroperazine is retained even after 20 h of continuous administration [45]. Thus, the β cell

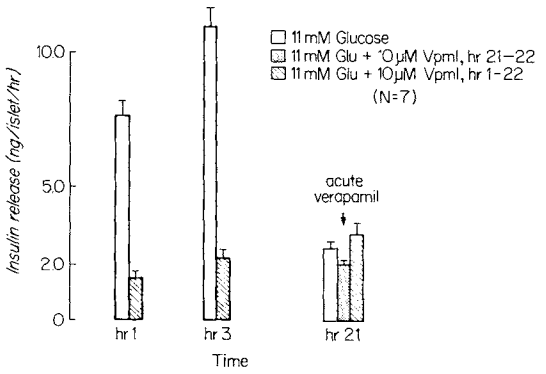

Fig. 6. Comparison of chronic vs. acute administration of verapamil on glucose-stimulated insulin secretion from isolated islets. Islets were continuously exposed to glucose, 11 mM. Verapamil inhibits insulin secretion during the first 3 h of chronic exposure but was no longer inhibitory at 21 h. Islets were still sensitive to verapamil if exposed for the first time at 21 h [data from 45].

has the ability to desensitize to both positive and negative regulators of cell function at the level of stimulus-secretion coupled activity. It is probable that α and δ, as well as other non-islet secretory cells, similarly maintain modulated secretory rates. The adaptive value of such homeostatic activity is clear. The underlying mechanisms, however, are difficult to disentangle but can be the basis of important future studies in islet physiology.

REFERENCES

1. Rossetti L, Giaccari A, DeFronzo RA: Diabetes Care 13:610–630, 1990.
2. Kosaka K, Kuzuya T, Akanuma Y, Hagura R: Diabetologia 18:23–28, 1980.
3. Grodsky GM: Diabetes 38:673–678, 1989.
4. Bolaffi JL, Bruno L, Heldt A, Grodsky GM: Endocrinology 122:1801–1809, 1988.
5. Hoenig M, MacGregor LC, Matschinsky FM: Am J Physiol 250:E502–E511, 1986.
6. Giroix MH, Serradas P, Portha B: Endocrinology 125: 1999–2007, 1989.
7. Purrello F, Vetri M, Gatta C, Gullo D, Vigneri R: Diabetes 38:1417–1422, 1989.
8. Voyles NR, Powell AM, Timmers KI, Wilkins SD, Bhathena SJ, Hansen C, Michaelis OE IV, Recant L: Diabetes 37:398–404, 1988.
9. Weir GC, Leahy JL, Bonner-Weir S: Diabetes Metab Rev 2:125–161, 1986.
10. Unger RH: Science 251:1200–1205, 1991.
11. Kergoat M, Bailbe D, Portha B: Diabetes 36:971–977, 1987.
12. Grill V, Westberg M, Ostenson CG: J Clin Invest 80:664–669, 1987.
13. MacDonald MJ, Fahien LA, McKenzie DI, Moran SM: Am J Physiol 259:E548–E554, 1990.

14. Marynissen G, Leclercq-Meyer V, Sener A, Malaisse WJ: Metabolism 39:87–95, 1990.
15. Timmers KI, Powell AM, Voyles NR, Solomon D, Wilkins SD, Bhathena S, Recant L: Diabetes 39:1436–1444, 1990.
16. Giroix M-H, Portha B, Bailbe D, Picon L: Diabetes 32:445–451, 1983.
17. Grill V, Rundfeldt M: Diabetes 35:44–51, 1986.
18. Portha B, Blondel O, Serradas P, Mc Evoy R, Giroix MH, Kergoat M, Bailbe D: Diabete Metabol 15:61–75, 1989.
19. Welsh N, Hellerstrom C: Endocrinology 126:1842–1848, 1990.
20. Vague P, Moulin JP: Metabolism 31:139–142, 1982.
21. Leahy JL, Weir GC: Diabetes 37:217–222, 1988.
22. Chen L, Komiya I, Inman L, McCorkle K, Alam T, Unger RH: PNAS 86:1367–1371, 1989.
23. Zucker P, Logothetopoulos J: Diabetes 24:194–200, 1975.
24. Grodsky GM: J Clin Invest 51:2047–2059, 1972.
25. Nesher R, Praiss M, Cerasi E: Acta Endocrinol 117:409–416, 1988.
26. Purrello F, Vetri M, Concetta G, Gullo D, Vigneri R: Diabetes 38:1417–1422, 1981.
27. Bolaffi JL, Heldt A, Lewis LD, Grodsky GM: Diabetes 35:370–373, 1986.
28. Curry DL: Endocrinology 118:170–175, 1986.
29. Lacy PE, Finke EH, Conant S, Naber S: Diabetes 25:484–493, 1976.
30. Nagamatsu S, Bolaffi, JL, Grodsky GM: Endocrinology 120:1225–1231, 1987.
31. Liang Y, Najafi H, Matschinsky FM: Diabetes 40(Suppl 1):705, 1991.
32. Brunstedt J, Chan SJ: BBRC 106:1383–1389, 1982.
33. Esrick DL, Sandler S: J Endocrinol 123:47–51, 1989.
34. Stefan Y, Meda P, Neufeld M, Orci L: J Clin Invest 80:175–183, 1987.
35. Chou HF, Ipp E: Diabetes 39:112–117, 1990.
36. Samols E, Stagner JI, Ewart RBL, Marks V: J Clin Invest 82:350–353, 1988.
37. Bolaffi JL, Rodd G, Ma YH, Grodsky GM: Endocrinology 126:1750–1755, 1990.
38. O'Connor MDL, Landahl H, Grodsky GM: Am J Physiol 238:R378–R389, 1980.
39. Zawalich WS, Zawalich KC, Shulman GI, Rossetti L: Endocrinology 126:253–260, 1990.
40. Weir GC, Leahy JL, Wilson J, Matschinsky FM: Diabetes 37:100A, 1988.
41. Portha B, Giroix MH, Serradas P, Welsh N, Hellerstrom C, Sener A, Malaisse WJ: Diabetes 37:1226–1233, 1988.
42. Marynissen G, Leclercq-Meyer V, Sener A, Malaisse WJ: Metabolism 39:87–95, 1990.
43. Hellman B: Diabetes Metab Rev 2:215–241, 1986.
44. Prentki M and Matschinsky FM: Physiol Rev 67:1185–1248, 1987.
45. Bolaffi JL, Rodd GG, Ma YH, Bright D, Grodsky GM: Endocrinology 129:2131–2138, 1991.
46. Wollheim CB, Kikuchi M, Renold AE, Sharp GWG: J Clin Invest 60:1165–1173, 1977.
47. Verspohl EJ, Kaiser P, Wahl M, Ammon HPT: Life Sci 43:209–219, 1988.
48. Purrello F, Vetri M, Vinci C, Gatta C, Buscema M, Vigneri R: Diabetes 39:97–99, 1990.
49. Joost S, Atwater I: In Atwater I, Rojas E, Soria B (eds): "Biophysics of the Pancreatic B-cell." New York: Plenum Press, pp 159–166, 1986.
50. Hughes SJ, Christie MR, Ashcroft SJH: Mol Cell Endocrinol 50:231–236, 1987.
51. Wolf BA, Easom RA, Hughes JH, McDaniel ML, Turk J.: Biochemistry 28:4291–4301, 1989.
52. Metz SA: Diabetes 37:3–7, 1988.
53. Zawalich WS: In Draznin B, Melmed S, LeRoith D (eds): "Molecular and Cellular Biology of Diabetes Mellitus," Vol 1 (Insulin Secretion) New York: Alan Liss, pp 69–80, 1989.
54. Laychock SG: Biochem J 216:101–106, 1983.
55. Ganesan S, Calle R, Zawalich K, Smallwood JI, Zawalich WS, Rasmussen H: PNAS 87:9893–9897, 1990.
56. Hallberg A: Acta Physiol Scand 128:267–276, 1986.
57. Peter-Reisch B, Fathi M, Schlegel W, Wollheim CB: J Clin Invest 81:1154–1161, 1988.
58. Zawalich WS, Zawalich KA, Shulman GI, Rossetti L: Endocrinology 126:253–260, 1990.
59. Easom RA, Landt M, Colca JR, Hughes JH, Turk J, McDaniel M: J Biol Chem 265:14938–14946, 1990.
60. Thams P, Capito K, Hedeskov CJ, Kofod H: Biochem J 265:777–787, 1990.
61. Persaud SJ, Jones PM, Howell SL: Biochim Biophys Acta 1091:120–122, 1991.
62. Thams P: Diabetes 40:82A, 1991.
63. Dunlop ME, Larkins RG: Biochem J 253:67–72, 1988.
64. Grodsky GM, Bolaffi JL: 14th International Diabetes Federation Congress, Washington DC, June 1991 (in press).
65. Frankel BJ, Kromhout JA, Imagawa W, Landahl H, Grodsky GM: Diabetes 27:365–369, 1978.