# Perspectives in Diabetes

# Triggering and Amplifying Pathways of Regulation of Insulin Secretion by Glucose

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Glucose stimulates insulin secretion by generating triggering and amplifying signals in β-cells. The triggering pathway is well characterized. It involves the following sequence of events: entry of glucose by facilitated diffusion, metabolism of glucose by oxidative glycolysis, rise in the ATP-to-ADP ratio, closure of ATP-sensitive K+ ($K_{ATP}$) channels, membrane depolarization, opening of voltage-operated $Ca^{2+}$ channels, $Ca^{2+}$ influx, rise in cytoplasmic free $Ca^{2+}$ concentration ($[Ca^{2+}]_i$), and activation of the exocytotic machinery. The amplifying pathway can be studied when β-cell $[Ca^{2+}]_i$ is elevated and clamped by a depolarization with either a high concentration of sulfonylurea or a high concentration of K+ in the presence of diazoxide ($K_{ATP}$ channels are then respectively blocked or held open). Under these conditions, glucose still increases insulin secretion in a concentration-dependent manner. This increase in secretion is highly sensitive to glucose (produced by as little as 1–6 mmol/l glucose), requires glucose metabolism, is independent of activation of protein kinases A and C, and does not seem to implicate long-chain acyl-CoAs. Changes in adenine nucleotides may be involved. The amplification consists of an increase in efficacy of $Ca^{2+}$ on exocytosis of insulin granules. There exists a clear hierarchy between both pathways. The triggering pathway predominates over the amplifying pathway, which remains functionally silent as long as $[Ca^{2+}]_i$ has not been raised by the first pathway; i.e., as long as glucose has not reached its threshold concentration. The alteration of this hierarchy by long-acting sulfonylureas or genetic inactivation of $K_{ATP}$ channels may lead to inappropriate insulin secretion at low glucose. The amplifying pathway serves to optimize the secretory response not only to glucose but also to nonglucose stimuli. It is impaired in β-cells of animal models of type 2 diabetes, and indirect evidence suggests that it is altered in β-cells of type 2 diabetic patients. Besides the available drugs that act on $K_{ATP}$ channels and increase the triggering signal, novel drugs that correct a deficient amplifying pathway would be useful to restore adequate insulin secretion in type 2 diabetic patients. *Diabetes* 49:1751–1760, 2000

P ancreatic β-cells synthesize insulin and secrete it at appropriate rates to maintain blood glucose levels within a relatively narrow range. Any alteration in their functioning has a profound impact on glucose homeostasis: excessive secretion of insulin causes hypoglycemia, and insufficient secretion leads to diabetes. This apparent simplicity of the β-cell's role sharply contrasts with the astonishing complexity of its regulation, which is ensured by an array of metabolic (glucose and other nutrients), neural, hormonal, and sometimes pharmacological factors.

The study of stimulus-secretion coupling in β-cells started in the 1960s and rapidly led to three key discoveries. First, glucose must be metabolized by β-cells to induce insulin secretion. This conclusion was based on the evidence that the insulinotropic effect of glucose is inhibited by agents that interfere with cellular metabolism and is mimicked only by metabolized sugars (1,2). Second, $Ca^{2+}$ has an essential role in insulin secretion. This was established by the demonstration that glucose does not increase insulin secretion in the absence of extracellular $Ca^{2+}$ (3,4). Third, pancreatic β-cells are electrically excitable. This was shown by the recording of action potentials in glucose-stimulated β-cells (5).

Glucose metabolism, electrical excitability, and $Ca^{2+}$ were the first elements of a puzzle that is still incomplete, even if the important features of the whole picture are beginning to appear clearly. Only one of these features will be developed in this article: the concept that glucose stimulation of insulin secretion involves the generation of both triggering and amplifying signals through distinct pathways. The generation of the triggering signal is well established and will be outlined only briefly, whereas the generation of the amplifying signal, which has been identified more recently, will be discussed in greater detail.

## THE TRIGGERING PATHWAY OF GLUCOSE-INDUCED INSULIN SECRETION

**Cytoplasmic $Ca^{2+}$ as triggering signal.** It is important to distinguish clearly between $Ca^{2+}$-independent events and events that require the presence of $Ca^{2+}$ (basal or elevated) but do not involve a change in $Ca^{2+}$ concentration. In insulin-secreting cells, of which the plasma membrane has been permeabilized (thus allowing partial control of the composition of the cytosol), exocytosis can be induced in a truly $Ca^{2+}$-independent manner by stable derivatives of GTP (6,7). This effect of GTP is also observed in voltage-clamped single
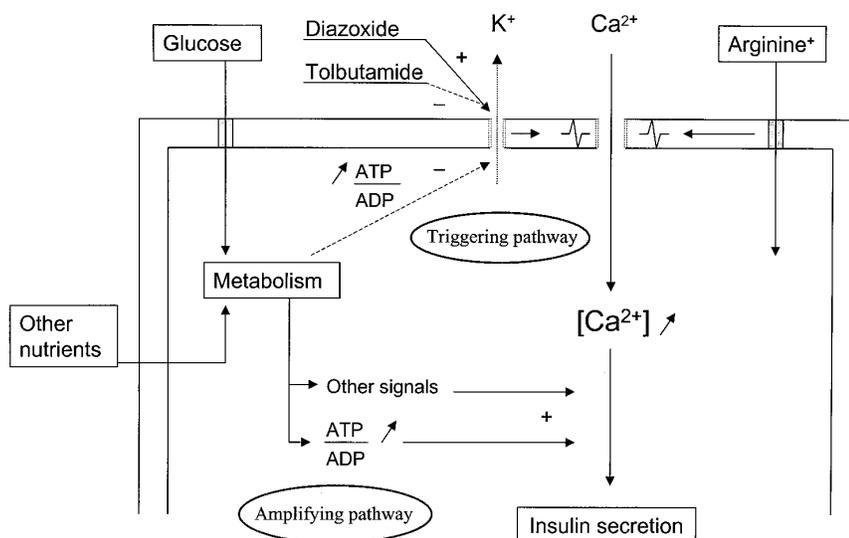
FIG. 1. Schematic representation of the triggering and amplifying pathways of the stimulation of insulin secretion by glucose. +, stimulation; −, inhibition.

β-cells in which secretion is monitored as increases in cell capacitance (8), which reflect fusion of the secretory granule membrane with the plasma membrane during exocytosis. In intact islets, glucose has been reported to increase insulin release in a concentration-dependent manner under conditions of stringent $Ca^{2+}$ depletion, but this effect is detected only during strong activation of both protein kinase A and protein kinase C (9). In our hands, however, the amount of insulin that can be released in the complete absence of $Ca^{2+}$ does not exceed a minor fraction of that released by appropriate controls in the presence of $Ca^{2+}$. Moreover, this $Ca^{2+}$-independent release shows no or only poor glucose dependence (10). Although the sequence of events leading to insulin secretion may include $Ca^{2+}$-independent steps, the physiological regulation by glucose is achieved through $Ca^{2+}$-dependent pathways.

The second messenger property of $Ca^{2+}$ rests in the possibility of changing the concentration of free cytoplasmic $Ca^{2+}$ ($[Ca^{2+}]_i$) very rapidly and markedly during stimulation. Like other cell types, unstimulated β-cells maintain a low $[Ca^{2+}]_i$ (50–100 nmol/l). There thus exist large gradients of $Ca^{2+}$ concentration between the cytoplasm and either the lumen of the endoplasmic reticulum (~2,000-fold) or the extracellular medium (~10,000-fold). Opening of $Ca^{2+}$ channels in the plasma membrane (e.g., by depolarization) or in the endoplasmic reticulum membranes (e.g., by inositol trisphosphate) allows rapid movements of $Ca^{2+}$ down the electrochemical gradient and causes increases in $[Ca^{2+}]_i$.

Under physiological conditions, glucose increases $[Ca^{2+}]_i$ in β-cells, and all maneuvers interfering with this rise impair the stimulation of insulin secretion. Direct evidence that cytoplasmic $Ca^{2+}$ triggers exocytosis of insulin granules has been provided by experiments using permeabilized islet cells (11,12) or capacitance recordings in single β-cells (13,14). Although progressive desensitization to $Ca^{2+}$ occurs in these preparations (7,15), probably because of the loss of essential cytoplasmic constituents, imposed oscillations of $[Ca^{2+}]_i$ are able to entrain oscillations of secretion in permeabilized cells, indicating that $Ca^{2+}$ has a minute-to-minute triggering

role (7). The mechanisms underlying the action of $Ca^{2+}$ are partially identified and have been reviewed elsewhere (16–19). The concept that cytoplasmic $Ca^{2+}$ serves as triggering signal in glucose-induced insulin secretion is widely accepted.

**Mechanisms of the triggering pathway.** $Ca^{2+}$ handling by intracellular organelles is influenced by (and can influence) glucose metabolism in β-cells. It certainly contributes to the fine regulation of $[Ca^{2+}]_i$ (20), but $Ca^{2+}$ influx through voltage-operated $Ca^{2+}$ channels is indisputably the major determinant of the glucose-induced $[Ca^{2+}]_i$ rise (Fig. 1).

Upon stimulation with glucose or other nutrients, the first response of β-cells is an acceleration of metabolism (21–23). Next, the $K^+$ conductance of the plasma membrane decreases (24–26), allowing a background current of a yet unidentified nature to move the potential away from the equilibrium potential for $K^+$ (i.e., to depolarize the membrane) (27,28). The decrease in $K^+$ conductance results from the closure of ATP-sensitive $K^+$ channels ($K_{ATP}$ channels) (29,30), the $K^+$ channels that dominate the resting membrane potential. These channels are tetramers of a complex of two proteins: a high-affinity sulfonylurea receptor (SUR1) and an inwardly rectifying $K^+$ channel (Kir 6.2). SUR1 endows the pore-forming Kir 6.2 with sensitivity to sulfonylureas and diazoxide, which respectively close and open the channel (Fig. 1). SUR1 also mediates the opening action of $Mg^{2+}$-ADP, whereas the closing action of ATP is on Kir 6.2 itself. Original references and extensive discussion of the channel structure and regulation can be found in recent review articles (31–33). It has not been proven that adenine nucleotides are the main or sole regulators of the channel in intact β-cells, but evidence that the ATP-to-ADP ratio changes over a wide range of glucose concentrations (34,35) supports their role.

When depolarization reaches the threshold of activation of voltage-operated $Ca^{2+}$ channels, these open, allowing the influx of $Ca^{2+}$ into the β-cell, down the electrical gradient (27,28). This opening of $Ca^{2+}$ channels is intermittent, oscillating with the membrane potential and therefore resulting in oscillations of $[Ca^{2+}]_i$ (36,37) that in turn trigger oscillations
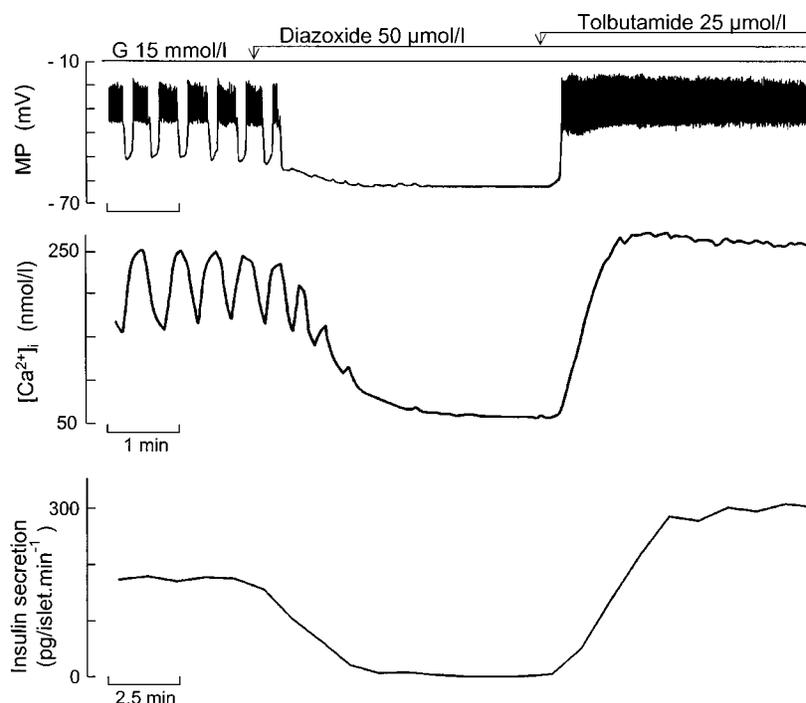
**FIG. 2. Demonstration of the essential role of $K_{ATP}$ channels in the stimulation of insulin secretion by glucose.** *Upper panel:* β-cell membrane potential (MP) recorded with an intracellular electrode in an intact mouse islet. *Middle panel:* cytoplasmic $[Ca^{2+}]_i$ in an intact mouse islet. *Lower panel:* insulin secretion by a batch of mouse islets; oscillations of secretion cannot be detected under the experimental conditions used. The perifusion medium contained 15 mmol/l glucose. Diazoxide and tolbutamide were added as indicated to open and close $K_{ATP}$ channels, respectively. Adapted from Gilon and Henquin (37).

of insulin secretion (38). Discussions of the oscillatory events in β-cells can be found elsewhere (39–41).

$K_{ATP}$ channels can thus be viewed as transducers of glucose-induced metabolic changes into biophysical changes. Their critical role in the stimulation of insulin secretion is easily demonstrated using agents that open or close the channels without interfering with glucose metabolism. When mouse islets are stimulated with 15 mmol/l glucose, β-cells display typical electrical activity, $[Ca^{2+}]_i$ oscillates, and insulin secretion is stimulated (Fig. 2). Opening $K_{ATP}$ channels with diazoxide causes membrane repolarization, lowering of $[Ca^{2+}]_i$, and inhibition of insulin secretion. Subsequent addition of tolbutamide to close the channels is followed by depolarization, resumption of electrical activity, rise in $[Ca^{2+}]_i$, and increase in insulin secretion (Fig. 2).

In summary, the consensus model explaining how glucose generates a triggering signal in β-cells involves the following steps: entry of glucose by facilitated diffusion, metabolism of glucose by oxidative glycolysis, rise in ATP-to-ADP ratio, closure of $K_{ATP}$ channels, membrane depolarization, opening of voltage-operated $Ca^{2+}$ channels, $Ca^{2+}$ influx, rise in $[Ca^{2+}]_i$, and activation of the exocytotic machinery (Fig. 1).

## THE AMPLIFYING PATHWAY OF GLUCOSE-INDUCED INSULIN SECRETION

The concept that glucose-induced insulin secretion involves more than one mechanism finds its roots in circumstantial observations that could not be fully interpreted when they were made. It has been markedly developed since 1992, when two independent studies showed that $K_{ATP}$ channels of

the β-cell membrane are not the only sites of control of insulin secretion by glucose (42,43).

**Glucose can increase insulin secretion when $K_{ATP}$ channels cannot be closed.** When $K_{ATP}$ channels are opened by diazoxide, the stimulation of insulin secretion by glucose is abrogated because the β-cell membrane does not depolarize and $[Ca^{2+}]_i$ does not increase. Another way to depolarize β-cells is to increase the concentration of extracellular $K^+$, a maneuver that simply shifts the equilibrium potential for $K^+$ to more positive values. Under these conditions, $Ca^{2+}$ influx and insulin secretion are stimulated, and neither of these effects is inhibited by diazoxide (44). The additional step made in 1992 was to show that glucose is still able to increase insulin secretion from islets depolarized with high $K^+$, although it is unable to close $K_{ATP}$ channels in the presence of diazoxide (42,43). We also demonstrated that this effect of glucose is concentration-dependent, requires metabolism, and does not involve any change in membrane potential or $[Ca^{2+}]_i$ (42), whereas Sato et al. (43) suggested that glucose metabolism is not required.

Using the same paradigm of $K^+$-induced depolarization in the presence of diazoxide, many laboratories have confirmed that glucose can increase insulin secretion independently of its action on $K_{ATP}$ channels in rodent islets (45–49), human islets (50), perfused rat pancreas (51), and insulin-secreting cell lines (52).

**Glucose can increase insulin secretion when $K_{ATP}$ channels are already completely closed.** By binding to SUR1, sulfonylureas close $K_{ATP}$ channels, depolarize the β-cell membrane, raise $[Ca^{2+}]_i$, and increase insulin secretion (Fig. 1). $K_{ATP}$ channels are unlikely to remain a site of
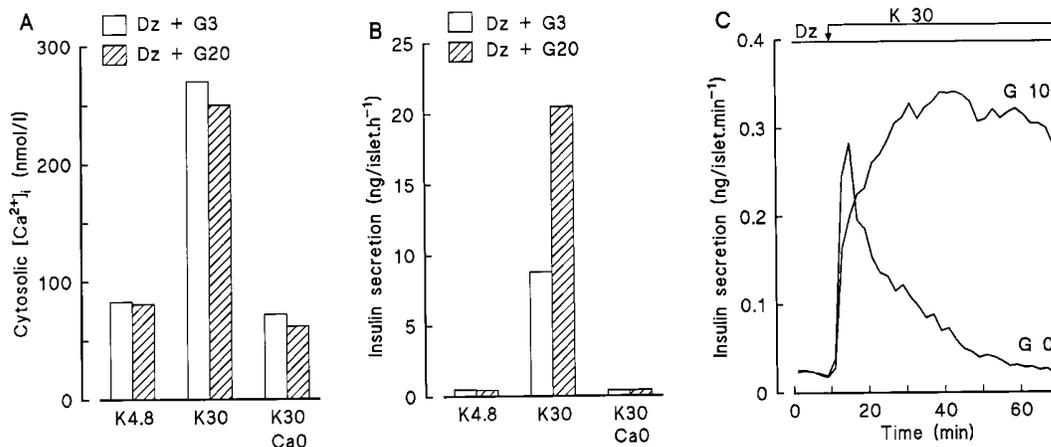
**FIG. 3. Glucose can increase insulin secretion independently of a closure of $K_{ATP}$ channels. All experiments were performed in the presence of diazoxide (Dz) to hold $K_{ATP}$ channels open. This prevented glucose (G) from increasing $[Ca^{2+}]_i$ ($A$) and stimulating insulin secretion ($B$) in the presence of 4.8 mmol/l $K^+$. Depolarization of β-cells with 30 mmol/l $K^+$ raised $[Ca^{2+}]_i$ and triggered insulin secretion at low (3 mmol/l) glucose. High (20 mmol/l) glucose slightly decreased $[Ca^{2+}]_i$ (by stimulating $Ca^{2+}$ uptake in the endoplasmic reticulum), but markedly augmented insulin secretion. This effect of glucose on insulin secretion was abolished when the $K^+$-induced $[Ca^{2+}]_i$ rise was prevented by omission of extracellular $CaCl_2$. $K^+$-induced insulin secretion is transient in the absence of glucose (G 0 mmol/l) and sustained in the presence of glucose (G 10 mmol/l) ($C$).**

regulation by glucose when they are all blocked by maximally effective concentrations of sulfonylureas. However, glucose has long been shown to increase insulin secretion under these conditions (53–57). In early studies, the phenomenon could not be investigated in enough detail to permit full interpretation, but the hypothesis that glucose might control insulin secretion by a mechanism not restricted to an inhibition of $K_{ATP}$ channels (56,57) eventually turned out to be correct.
**Both approaches uncover an amplifying pathway.** In the presence of diazoxide and a control concentration of $K^+$ (4.8 mmol/l), 20 mmol/l glucose is virtually without effect on basal β-cell $[Ca^{2+}]_i$ and insulin secretion (Fig. 3$A$ and $B$). Depolarizing the membrane with 30 mmol/l $K^+$ increases $[Ca^{2+}]_i$ and triggers insulin secretion at low (3 mmol/l) glucose. High glucose slightly lowers this elevated $[Ca^{2+}]_i$ but markedly augments insulin secretion. This effect on secretion is totally suppressed when the $K^+$-induced $[Ca^{2+}]_i$ rise is prevented by omission of extracellular $Ca^{2+}$. The $K_{ATP}$ channel–independent effect of glucose on insulin secretion is thus $Ca^{2+}$-dependent ($[Ca^{2+}]_i$ has to be elevated), but is not mediated by any further rise in $[Ca^{2+}]_i$ (42,58).

When mouse islets are perifused with a supramaximal concentration of glibenclamide (2 μmol/l), $[Ca^{2+}]_i$ is elevated and insulin secretion is stimulated even in 3 mmol/l glucose (Fig. 4$A$ and $B$). When the glucose concentration is raised to 20 mmol/l, $[Ca^{2+}]_i$ transiently decreases before returning to control values, whereas insulin secretion markedly increases after a small initial drop. The final lowering of glucose to 3 mmol/l is accompanied by a minor rise in $[Ca^{2+}]_i$ and a marked decrease in insulin secretion (59). It is evident that more insulin is secreted at high glucose levels than at low glucose levels, although $[Ca^{2+}]_i$ is similar.

Fig. 4$C$ shows the relationship between insulin secretion and islet $[Ca^{2+}]_i$ when the islets are depolarized by high $K^+$ alone, by high $K^+$ in the presence of diazoxide, or by sulfonylureas (59). The excellent correlation found at both 3 and 20 mmol/l glucose strongly supports the crucial role of $Ca^{2+}$ as triggering signal. However, the essential message of Fig. 4$C$ is that, regardless of experimental conditions, the

secretory response to a given $[Ca^{2+}]_i$ is larger in high than in low glucose. Similar correlations are obtained when stepwise changes in $[Ca^{2+}]_i$ are imposed in $K^+$-depolarized islets by changes in extracellular $Ca^{2+}$ (60). Our whole series of studies (42,58–62) establishes beyond doubt that the amplifying pathway of insulin secretion leads to an increase in the efficacy of cytosolic $Ca^{2+}$ in the secretory process.

Studying the amplifying pathway in intact cells is not without pitfalls. Whereas exclusion of an action through $K_{ATP}$ channels is relatively easy, one cannot simply assume that glucose or other test substances do not affect the magnitude of the triggering signal. For example, in the presence of diazoxide and only 20 mmol/l $K^+$, high glucose only marginally increases insulin secretion because of the substantial lowering of $[Ca^{2+}]_i$ that it also produces (59). In this situation, the amplifying action would escape detection in the absence of $[Ca^{2+}]_i$ measurements. The opposite problem is encountered when β-cells are depolarized by high $K^+$ in the absence of diazoxide. Under these conditions, a rise in glucose concentration induces a slow monophasic increase in insulin secretion that was recognized 25 years ago and ascribed to a stimulation of $Ca^{2+}$ influx through reactivated voltage-operated $Ca^{2+}$ channels (63,64). More recent studies have shown that a small electrical activity indeed appears in β-cells (42) and that $[Ca^{2+}]_i$ slightly increases (59) under these conditions. In this situation, the increase in insulin secretion produced by glucose cannot be ascribed entirely to the amplifying pathway (59).
**Mechanisms of the amplifying pathway.** There is no dispute that the closure of $K_{ATP}$ channels and the subsequent rise in $[Ca^{2+}]_i$ induced by glucose require metabolism of the sugar in β-cells. In contrast, it has been proposed that the amplifying effect of glucose is weakly, if at all, dependent on glucose metabolism, but involves direct recognition of the glucose molecule (43,65). However, the tools used to assess the possible role of a glucoreceptor are not specific (66), and the high glucose sensitivity of the amplifying pathway (see below) can be mistaken for an independence of metabolism. We (42,58,66,67) and others (45–47) have shown that the amplifying pathway is activated by all metabolized nutrients and
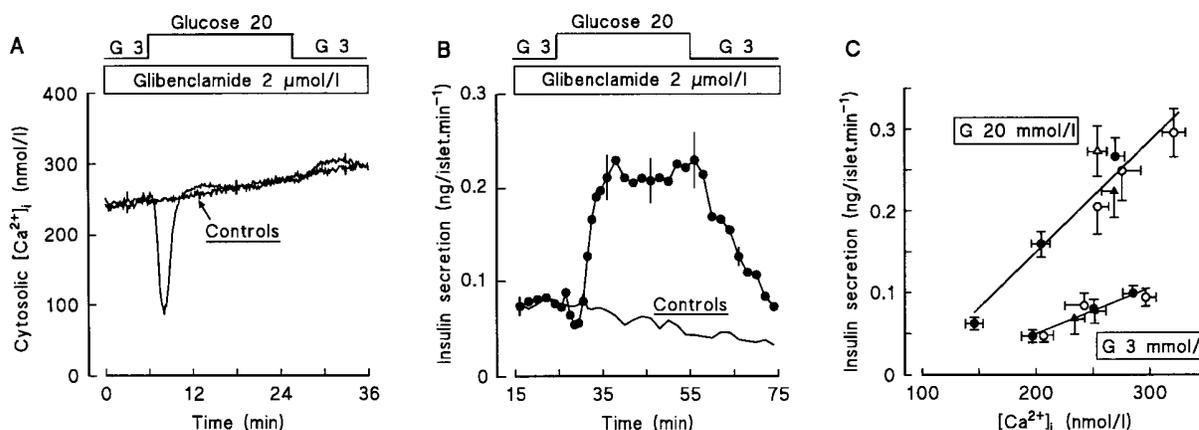
FIG.4. Glucose amplifies the effect of $Ca^{2+}$ on exocytosis. *A, B:* All $K_{ATP}$ channels in β-cells were blocked by perifusing mouse islets with a very high concentration of glibenclamide (2 µmol/l). The glucose (G) concentration was kept at 3 mmol/l throughout (controls) or raised to 20 mmol/l as indicated (tests). The transient fall in $[Ca^{2+}]_i$ induced by high glucose is attributed to uptake of $Ca^{2+}$ by the endoplasmic reticulum and partial repolarization of the plasma membrane (20; unpublished data). In the steady state, high glucose markedly increased insulin secretion, although $[Ca^{2+}]_i$ was not changed. *C:* Correlations between $[Ca^{2+}]_i$ and insulin secretion in mouse islets depolarized with high $K^+$ or sulfonylureas in the presence of 3 or 20 mmol/l glucose: 20, 25, or 30 mmol/l $K^+$ without (○) or with (●) diazoxide, 500 µmol/l tolbutamide (△), or 2 µmol/l glibenclamide (▲). Adapted from Sato et al. (59).

requires glucose metabolism. In this context, it is pertinent to emphasize that islet glucose metabolism is not altered under the conditions in which the amplifying pathway is often studied (250 µmol/l diazoxide, 30 mmol/l KCl, and 1–2.5 mmol/l $CaCl_2$) (62,68).

The second messenger issued from glucose metabolism has not been identified with certainty, but good correlations exist between islet adenine and guanine nucleotides and glucose-induced insulin secretion through the amplifying pathway (34,58). Gradually lowering the ATP-to-ADP ratio in mouse islets by increasing concentrations of mitochondrial poisons progressively inhibits insulin secretion even when measures are taken to prevent any decrease in $[Ca^{2+}]_i$ (67). Thus, ATP has effects on insulin secretion at steps distal to the production of the triggering signal. Potentiation of $Ca^{2+}$-induced exocytosis by cytoplasmic ATP is also observed in experiments monitoring secretion by capacitance changes in single β-cells (69,70). Glucose-induced changes in the ATP-to-ADP ratio are thus plausible candidates for control of both pathways of stimulation of insulin secretion.

Although the rise in $[Ca^{2+}]_i$ produced by high $K^+$ is sustained (58), the concomitant secretion of insulin is only transient when the islets are perifused with a glucose-free medium (Fig. 3*C*). The presence of glucose, even in low concentrations (58), strikingly transforms this monophasic response into a sustained secretion of insulin. In permeabilized islet cells stimulated by fixed elevated $Ca^{2+}$, insulin release is also transient because of a progressive desensitization to the high concentrations of $Ca^{2+}$ used (7,15). The desensitization appears to involve a decrease in the activity of calmodulin-dependent protein kinase II (71). This kinase, which is known to play an important role in insulin secretion (18), could participate in the amplifying action of glucose. Mobilization of insulin granules from reserve to release pools might underlie this change in secretory pattern.

The use of phosphatase inhibitors has suggested that both phosphorylation and dephosphorylation of certain β-cell proteins are involved in the amplifying pathway (72). On the basis of capacitance measurements in single β-cells, it has

been proposed that the action of ATP on exocytosis is mediated by the sequential activation of adenylate cyclase and protein kinase A (70). However, experiments using intact islets indicate that cAMP interacts with but does not mediate glucose stimulation of insulin secretion through the amplifying pathway (58,66,73). Several pieces of evidence from two laboratories (58,66,74) have established that the amplifying action of glucose does not involve the phospholipase C–protein kinase C pathway in the mouse. Discordant conclusions have been reached for the rat β-cell (65,74,75), but the most thorough study of the question also concluded that phospholipase C activation cannot be the major cause of glucose amplification of insulin secretion (75).

The possible contribution of several other messengers has been evaluated and rejected. These include production of arachidonic acid by phospholipase $A_2$, the NO-cGMP pathway, and phosphatidylinositol 3-kinase (66). It has recently been proposed that, besides ATP, glutamate derived from mitochondrial α-ketoglutarate could also control $Ca^{2+}$-induced exocytosis of insulin granules (76). This interesting hypothesis is currently under investigation.

The role of long-chain acyl-CoAs is controversial. Their concentration increases in glucose-stimulated insulin-secreting cells and much more so during stimulation with fatty acids (77). They have been shown to augment $Ca^{2+}$-induced insulin secretion in permeabilized cells (78). In our model of mouse islets, exogenous fatty acids do not mimic the amplifying action of glucose (66) and, in contrast to the latter, increase $[Ca^{2+}]_i$ in β-cells depolarized with 30 mmol/l $K^+$ in the presence of diazoxide (79). In rat islets, fatty acids increase $K^+$-induced insulin secretion, but this effect is not as sustained as that of glucose and requires pre-exposure of the islets to fatty acids under stringent $Ca^{2+}$-free conditions (80). The lack of $[Ca^{2+}]_i$ measurements makes interpretation of this study problematic.

**Hierarchy between triggering and amplifying pathways.** The concentration dependency of glucose-induced insulin secretion displays a sigmoidal shape, with threshold, half-maximal, and maximal concentrations of about 7, 15, and
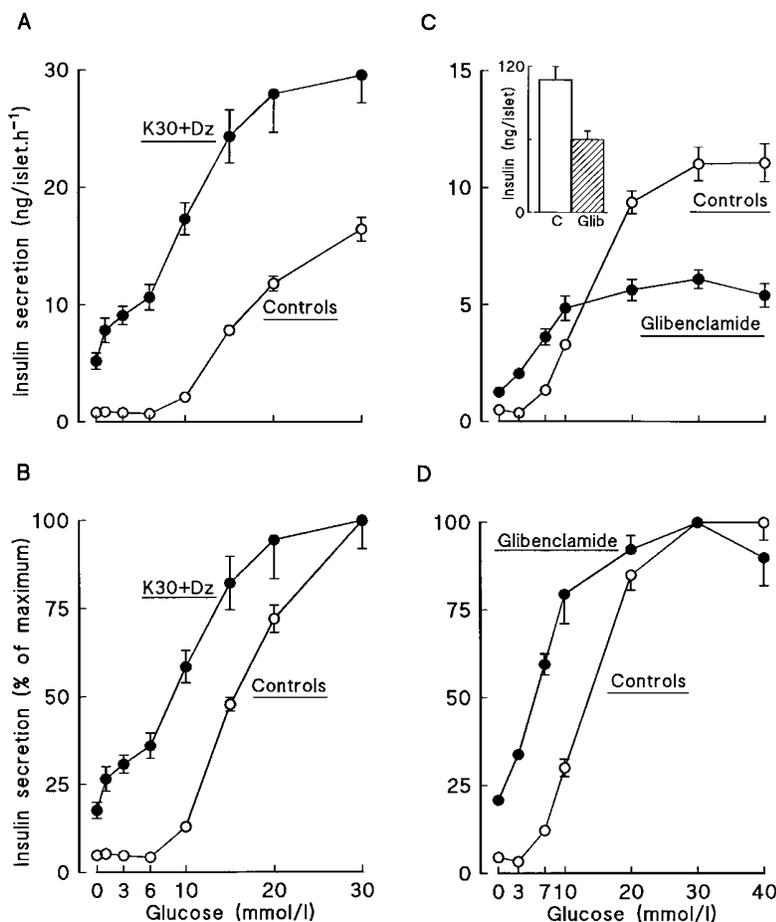
**FIG. 5. Concentration dependency of glucose-induced insulin secretion. In control islets (4.8 mmol/l K$^+$, no diazoxide or sulfonylurea) both pathways are operative. In test islets, the triggering signal ([Ca$^{2+}$]$_i$) is clamped (or almost) by K$^+$ plus diazoxide or by glibenclamide (42,58,61), and the effect of glucose on secretion is exerted through the amplifying pathway. *A, B:* Freshly isolated mouse islets, perifused with a medium containing diazoxide and various glucose concentrations, were stimulated for 1 h with 30 mmol/l K$^+$. Insulin secretion was integrated over the whole period of stimulation and expressed as absolute values (*A*) or percentage of the maximum response (*B*). The insulin content of both groups of islets was similar, but the triggering signal ([Ca$^{2+}$]$_i$) was larger in test than in control islets (not shown; 42,58), which explains the larger absolute responses. Adapted from Gembal et al. (58). *C, D:* Mouse islets were cultured for 18 h without (controls) or with 10 nmol/l gliben-clamide (therapeutic concentration), before being incubated for 60 min in the presence of various glucose concentrations without glibenclamide. The figure shows insulin secreted during that hour, as absolute values (*C*) or percentage of maximum (*D*). Under these conditions, [Ca$^{2+}$]$_i$ is already elevated in low glucose but can still be increased slightly by high glucose (not shown; 61). The triggering signal thus remains partially under control of glucose. The lower maximal response can be explained by the lower insulin content of the islets after culture with gliben-clamide (*inset*). Adapted from Anello et al. (61).**

30 mmol/l in mouse islets (Fig. 5). The threshold corresponds to the glucose concentration required to depolarize the β-cell membrane to the potential where voltage-operated Ca$^{2+}$ channels start to open and thus the triggering signal starts to be produced. In contrast, the concentration dependency of the amplifying pathway (studied in the presence of diazoxide and 30 mmol/l K$^+$) is hyperbolic, with half-maximal and maximal effects produced by 8–10 and 30 mmol/l glucose respectively (Fig. 5*A* and *B*). The distinct dependence of the two pathways on metabolism also becomes evident when mitochondrial ATP production is impaired pharmacologically. Thus, the rise in [Ca$^{2+}$]$_i$ in β-cells is impeded by much smaller decreases in ATP-to-ADP ratio than is the action of Ca$^{2+}$ on exocytosis (67).

Low concentrations of glucose (1–6 mmol/l) can thus influence insulin secretion, but their influence manifests itself only when a triggering signal has been produced (42,58). This establishes a clear hierarchy between the two path-

ways: the amplifying pathway remains functionally silent as long as the triggering pathway has not depolarized the membrane and raised [Ca$^{2+}$]$_i$. The amplifying pathway serves to optimize the secretory response induced by the triggering signal. This hierarchy between the two pathways ensures that no insulin is inappropriately secreted in the presence of low glucose concentrations. It is altered by genetic alterations of K$_{ATP}$ channels (see below). The hierarchy may also be altered in patients treated with long-acting sulfonylureas. The experiments shown in Fig. 5*C* compare secretion of insulin by control mouse islets to that by islets cultured for 18 h with a low therapeutic (10 nmol/l) concentration of glibenclamide. Under these conditions, β-cell [Ca$^{2+}$]$_i$ is elevated at low glucose and the major effect on secretion occurs at low concentrations (61). In vivo, a drug like glibenclamide may decrease or suppress the ability of glucose to control insulin secretion through the triggering pathway and restrict its action to the amplifying pathway.

**Physiological significance of the amplifying pathway.**
The existence of the amplifying pathway can easily be demonstrated under artificial conditions that clamp β-cell $[Ca^{2+}]_i$. It is much more difficult to determine what fraction of glucose-induced insulin secretion should be ascribed to one or the other pathway under physiological conditions; thus, any change in glucose concentration (above the threshold) affects both signals simultaneously. Several pieces of indirect evidence, however, support the importance of the amplifying pathway.

First, when controlled $[Ca^{2+}]_i$ oscillations, similar to those occurring spontaneously during glucose stimulation, are imposed by repetitive depolarizations of β-cells with high $K^+$, the resulting oscillations of insulin secretion increase in amplitude with the concentration of glucose (81). Second, during stimulation of mouse islets with glucose alone, a rise from a stimulatory to an even higher concentration increases the duration of the spontaneous $[Ca^{2+}]_i$ oscillations without changing their amplitude, whereas the concomitant oscillations of insulin secretion increase in both duration (triggering signal) and amplitude (amplifying pathway) (82). Third, when a similar oscillatory triggering signal is produced by a pharmacological agent (addition of 5 μmol/l tolbutamide to 10 mmol/l glucose) or by a rise in glucose (from 10 to 15 mmol/l), insulin secretion is greater at the higher glucose concentration (83). The amplifying pathway is thus operative when the triggering signal, $[Ca^{2+}]_i$, oscillates.

The amplifying pathway permits a $[Ca^{2+}]_i$ rise in β-cells to induce a sustained secretion of insulin (Fig. 3*C*). It therefore appears to be a major contributor to the second phase of nutrient-induced insulin secretion (45,58,84). It is still unclear whether the enlargement of the pool of releasable insulin granules results from a mobilization of granules toward the sites of exocytosis or a modification of the properties of already adequately situated granules.

Isolated β-cells are functionally heterogeneous, and the increase in various β-cell responses with increasing glucose concentrations has been attributed to the recruitment of more and more β-cells into an active state (85). However, the association of β-cells within islets may reduce this functional heterogeneity (86). Thus, in clusters of β-cells, all cells start to respond with a $[Ca^{2+}]_i$ rise between 6 and 10 mmol/l glucose (87). Even though the recruitment occurs over a narrow range of glucose concentrations, which results in a rather homogeneous triggering signal in individual β-cells within intact islets (36,37), insulin secretion might still be heterogeneous (88) if the production of amplifying signals is variable between β-cells.

**Amplifying pathway in genetically modified β-cells.**
Overexpression of a dominant-negative form of Kir 6.2 specifically in β-cells caused a functional inactivation of $K_{ATP}$ channels and produced a model of neonatal hypoglycemia followed by rapid development of diabetes caused by β-cell death (89). How glucose influences insulin secretion from these cells could not be investigated. Mice lacking $K_{ATP}$ channels have recently been generated by knockout of either Kir 6.2 (90) or SUR1 (91). Except for mild neonatal and fasting hypoglycemia in SUR1 knockout mice, both models have nearly normal blood glucose levels. As expected with suppression of $K_{ATP}$ channels, β-cells from both types of mice are depolarized and $[Ca^{2+}]_i$ is elevated even in low glucose. Upon stimulation by high glucose, there occurs a minor early increase (Kir 6.2 knockout) or a progressive delayed rise (SUR1 knockout) in insulin secretion, while $[Ca^{2+}]_i$ slightly decreases (90,92).

Spontaneous loss of function of $K_{ATP}$ channels (generally due to mutations of SUR1) is responsible for one form of persistent hyperinsulinemic hypoglycemia of infancy (31–33). These infants cannot readily restrain insulin secretion in the face of low blood glucose because their β-cells are continuously depolarized with elevated $[Ca^{2+}]_i$. A β-cell line (NES2Y) derived from one of these patients has permitted studies of stimulus-secretion coupling. These cells secrete large amounts of insulin in the presence of 0.5 mmol/l glucose, and a slight (50%) increase is produced by high glucose (93).

The amplifying pathway of glucose-induced insulin secretion thus exists in these β-cells with engineered or natural inactivation of $K_{ATP}$ channels. However, it appears surprisingly less effective than in normal islets with a pharmacological exclusion of $K_{ATP}$ channels. Several explanations can be proposed. First, $K_{ATP}$ channels may have a second function, independent of the membrane potential regulation and implicated in the amplifying pathway. It should be borne in mind that this pathway is independent of the open or closed state of the channels (59). Second, these chronically stimulated β-cells might not contain enough insulin to develop a strong secretory response in vitro. Third, several effector systems, for example calmodulin-dependent protein-kinase II (71), may have been downregulated in these β-cells whose $[Ca^{2+}]_i$ is continuously elevated. Detailed studies of stimulus-secretion coupling in these models should resolve the question.

**Amplifying pathway in animal models of type 2 diabetes.**
The technique of depolarization by high $K^+$ in the presence of diazoxide has been used to investigate the amplifying pathway of glucose-induced insulin secretion in β-cells from various models of type 2 diabetes. Alterations have been identified in islets from diabetic GK (Goto-Kakizaki) rats (51,94), from glucose-intolerant hybrids of diabetic GK and control Wistar rats (95), and from rats rendered glucose-intolerant by neonatal malnutrition followed by sucrose-feeding (96). The defect in glucose-induced insulin secretion in these animals is ascribed to anomalies of both the triggering pathway (impaired $K_{ATP}$ channel closure) and the amplifying pathway, which is compatible with an upstream alteration of the glucose recognition system.

**Amplifying pathway in humans.** The amplifying pathway is operative in isolated human islets (50; unpublished observations). For obvious reasons, the experimental conditions used to study it in vitro cannot be used in vivo. However, early studies of insulin secretion in humans have made a distinction between initiating and potentiating actions of glucose (97,98). The increase in plasma insulin levels brought about by nonglucose stimuli, such as tolbutamide or arginine, is augmented by the level of glycemia. The phenomenon has been referred to as the potentiating action of glucose as opposed to its initiating action, i.e., the rise in plasma insulin that glucose itself causes. The amplifying pathway is certainly involved in the phenomenon, but does not explain it entirely. Thus, the triggering signal produced by these nonglucose stimuli (at least when they are used at low concentrations comparable to those reached in vivo) also increases with the glucose concentration (99,100). The augmentation of their insulinotropic action by glucose most likely involves changes in both triggering and amplifying signals.

In type 2 diabetic patients, acute stimulation of insulin secretion by glucose is impaired, and this defect can be compensated for by sulfonylureas and other drugs directly closing $K_{ATP}$ channels. However, the potentiating action of glucose is also defective (101), and none of the currently available drugs are able to correct this defect. Thus, an ideal drug or combination of drugs should aim at both triggering and amplifying pathways, but it would be unwise to stimulate the amplifying pathway strongly if the generation of the triggering signal can no longer be controlled by glucose (e.g., in the presence of long-acting sulfonylureas). The patients would incur a serious risk of inappropriate insulin secretion and therefore hypoglycemia. The natural hierarchy between the two pathways is a safeguard and should not be reversed pharmacologically.

## TERMINOLOGY

Because the two studies published in 1992 specifically aimed at studying "whether glucose can control insulin secretion independently from its action on K⁺-ATP channels," it is becoming popular to refer to the "$K_{ATP}$ channel–dependent" and "$K_{ATP}$ channel–independent" pathways. In this article, I chose to use the terms "triggering pathway" and "amplifying pathway." The two terminologies are not completely equivalent. The term $K_{ATP}$ channel–dependent pathway is precise and restrictive. It does not include any other mechanism by which glucose might change the magnitude of the triggering signal, e.g., by an action on voltage-operated $Ca^{2+}$ channels (102) or $Ca^{2+}$-ATPases. Conversely, the term $K_{ATP}$ channel–independent pathway is vague, saying nothing about where and how glucose might act.

The terminology that I propose has several advantages. First, the terms triggering and amplifying immediately define both the functional role and the hierarchy of the two pathways. Second, they leave open the possibility that intracellular (mitochondrial) $K_{ATP}$ channels (103,104) serve a function in insulin secretion that is distinct from that of their congeners in the plasma membrane. Third, confusion is avoided when the effects of glucose are compared to those of certain other nutrients. Arginine depolarizes β-cells because of its transport in a positively charged form (105). It therefore produces a triggering signal (rise in $[Ca^{2+}]_i$) through a $K_{ATP}$ channel–independent pathway, but does not produce any amplifying signal (Fig. 1). Fourth, the parallelism between the effects of glucose and those of agents acting on membrane receptors becomes more clear. An agent such as acetylcholine always acts through $K_{ATP}$ channel–independent pathways. However, it produces or augments the triggering signal (either by $Ca^{2+}$ mobilization or $Na^+$-dependent depolarization) and produces amplifying signals that act on protein kinase C to increase the efficacy of $Ca^{2+}$ on exocytosis.

The concept of triggering and amplifying signals is unambiguous and coherent. It is applicable to all β-cell stimuli, whether they produce only one or both signals and regardless of the relative proportion and mechanisms of this production. It is also applicable to inhibitors of insulin secretion.

## ACKNOWLEDGMENTS

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