Dual mechanism of the potentiation by glucose of insulin secretion induced by arginine and tolbutamide in mouse islets

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Ishiyama, Nobuyoshi, Magalie A. Ravier, and Jean-Claude Henquin. Dual mechanism of the potentiation by glucose of insulin secretion induced by arginine and tolbutamide in mouse islets. Am J Physiol Endocrinol Metab 290: E540–E549, 2006.—Glucose induces insulin secretion (IS) and also potentiates the insulin-releasing action of secretagogues such as arginine and sulfonylureas. This potentiating effect is known to be impaired in type 2 diabetic patients, but its cellular mechanisms are unclear. IS and cytosolic Ca2+ concentration ([Ca2+]i) were measured in mouse islets during perfusion with 3–15 mmol/l glucose (G3–G15, respectively) and pulse or stepwise stimulation with 1–10 mmol/l arginine or 5–250 μmol/l tolbutamide. In G3, arginine induced small increases in [Ca2+]i, but no IS. G7 alone only slightly increased [Ca2+]i, and IS but markedly potentiated arginine effects on [Ca2+]i, which resulted in significant IS (already at 1 mmol/l). For each arginine concentration, both responses further increased at G10 and G15, but the relative change was distinctly larger for IS than [Ca2+]i. At all glucose concentrations, tolbutamide dose dependently increased [Ca2+]i and IS with thresholds of 25 μmol/l for [Ca2+]i, and 100 μmol/l for IS at G3 and of 5 μmol/l for both at G7 and above. Between G7 and G15, the effect of tolbutamide on [Ca2+]i increased only slightly, whereas that on IS was strongly potentiated. The linear relationship between IS and [Ca2+]i, at increasing arginine or tolbutamide concentrations became steeper as the glucose concentration was raised. Thus glucose augmented more the effect of each agent on IS than that on [Ca2+]i. In conclusion, glucose potentiation of arginine- or tolbutamide-induced IS involves increases in both the rise of [Ca2+]i, and the action of Ca2+ on exocytosis. This dual mechanism must be borne in mind to interpret the alterations of the potentiating action of glucose in type 2 diabetic patients.

insulin release; sulfonylureas; glucose potentiation; β-cell cytosolic calcium ion; type 2 diabetes

CHANGES IN THE GLUCOSE CONCENTRATION directly control insulin secretion and also modulate the insulin-releasing effects of other secretagogues (19, 40). This second action of glucose can manifest itself as an unmasking of the insulin-releasing properties of a substance (glucose dependence) or as an increase in the magnitude of the insulin response to that substance (glucose potentiation). Physiologically, the phenomenon prevents the hypoglycemia that nonglucose stimuli might cause if they were fully active at low glucose and augments the β-cell response in frequent situations of mixed stimulation. In type 2 diabetic patients, the ability of glucose to potentiate insulin secretion evoked by secretagogues such as arginine or tolbutamide is impaired (7, 11, 12, 18, 32, 38, 48). Surprisingly, this pathophysiologically important property of glucose has not been mechanistically explained.

Glucose stimulation of insulin secretion results from the activation of two signaling pathways set in operation by an acceleration of metabolism in β-cells (26). The triggering pathway involves closure of ATP-sensitive K+ channels (KATP channels), membrane depolarization, Ca2+ influx through voltage-dependent Ca2+ channels, and a rise in the cytosolic Ca2+ concentration ([Ca2+]i; see Refs. 1, 15, 41, 43, 45). However, the triggering signal is poorly effective alone. An amplifying pathway, using as-yet incompletely identified signals, augments insulin secretion without increasing [Ca2+]i further. This amplifying pathway, also known as the KATP channel-independent pathway, thus augments the efficacy with which Ca2+ induces exocytosis of insulin granules (26, 30, 45).

Arginine and tolbutamide are prototypic insulin secretagogues whose effects are not secondary to a stimulation of energy metabolism in β-cells. They share the property of increasing the triggering signal, [Ca2+]i, but do so by different mechanisms. Arginine is only slowly metabolized in β-cells, and none of the products generated by this metabolism, including NO, appears to play a positive role in the stimulation of insulin secretion (6, 21, 47). In particular, arginine does not serve as a fuel and does not accelerate the metabolism of glucose or endogenous nutrients, and, therefore, does not increase the energy state of β-cells (6, 47). Arginine is positively charged at physiological pH and depolarizes β-cells because its entry in the cell is electrogenic (8, 23, 44). This depolarization stimulates Ca2+ influx through voltage-dependent Ca2+ channels, leading to a rise in [Ca2+]i, that triggers or augments insulin secretion (15, 16, 35, 44). Tolbutamide, like all sulfonylureas, binds to sulfonurea receptor 1, the regulatory subunit of KATP channels, thereby closing the channels and leading to β-cell depolarization, Ca2+ influx, and a rise in [Ca2+]i (1, 15, 43).

To investigate the mechanisms by which glucose potentiates insulin secretion induced by arginine or tolbutamide, we measured the effects of various concentrations of the amino acid or the sulfonylurea on insulin secretion and [Ca2+]i, in mouse islets perfused in the presence of different glucose concentrations. Because tolbutamide also potentiates arginine-induced insulin secretion in vivo (39, 40), we compared the effects of the amino acid in the presence of the sulfonylurea with those in the presence of glucose. Our specific aim was to determine whether glucose potentiation is achieved by augmenting the triggering signal or by amplifying the efficacy of Ca2+ on secretion. In previous studies, the amplifying action of glucose...
was characterized under conditions preventing changes in the triggering signal, i.e., when β-cell [Ca\textsuperscript{2+}] was clamped (26). This straightforward approach cannot be used to determine whether glucose potentiation of arginine- or tolbutamide-induced insulin secretion involves a greater rise in [Ca\textsuperscript{2+}], (triggering pathway), a larger response to a given [Ca\textsuperscript{2+}] rise (amplifying pathway), or both. The only possible approach is to compare [Ca\textsuperscript{2+}] and insulin secretion changes induced by arginine or tolbutamide at different glucose concentrations.

**MATERIALS AND METHODS**

**Preparation and solutions.** The research project was approved by, and the experiments were conducted in accordance with, the guidelines of the “Commission d’Ethique et d’Experimentation Animale” of the University of Louvain Faculty of Medicine. Female NMRI mice from a local colony were killed by decapitation. Islets were then aseptically isolated by collagenase digestion of the pancreas, selected by hand-picking, and cultured for 1 day in RPMI 1640 medium containing 10 mmol/l glucose (5). The control medium used for islet isolation contained (mmol/l) 120 NaCl, 4.8 KCl, 2.5 CaCl\textsubscript{2}, 1.2 MgCl\textsubscript{2}, 24 NaHCO\textsubscript{3}, 10 glucose, and 1 mg/ml BSA. It was gassed with 94% O\textsubscript{2}-6% CO\textsubscript{2} to maintain a pH of 7.4. The same medium was used for all experiments, after adjustment of the glucose concentration and addition of the studied substances. Tolbutamide was from Sigma (St. Louis, MO); l-arginine monohydrochloride and other reagents were from Merck (Darmstadt, Germany).

**Measurements of insulin secretion.** After culture, the islets were preincubated for 1 h at 37°C in control medium containing 10 mmol/l glucose. Batches of 25 islets (45 islets for experiments in 3 mmol/l glucose) were then placed in small chambers of a perifusion system (25). Effluent samples were usually collected at 2-min intervals (at 1-min intervals in one experimental series) and saved until insulin assay with rat insulin as a standard (20). Each protocol was repeated five to six times with islets from different preparations.

**Measurements of [Ca\textsuperscript{2+}].** After culture, the islets were loaded with fura PE3 during 2 h of preincubation at 37°C in control medium containing 10 mmol/l glucose and 2 μmol/l fura PE3-AM. The islets were then placed in the perifusion chamber of a microspectrofluorimetric system that has been described in detail previously (15). Each protocol was tested in 10–15 islets from at least three separate preparations.

**Data presentation.** The results of [Ca\textsuperscript{2+}] and insulin secretion experiments are shown by mean traces ± SE. Because of technical constraints, [Ca\textsuperscript{2+}] experiments cannot be as long as insulin secretion experiments. Therefore, in the experiments testing four concentrations of arginine or tolbutamide, the application of the test substance was two times shorter in [Ca\textsuperscript{2+}] than in insulin measurements: 5 vs. 10 min.

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Fig. 1. Effects of arginine pulses on cytosolic Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}; A and C) and insulin secretion (B and D) in mouse islets. Islets were perifused throughout with 3, 7, 10, or 15 mmol/l glucose (G3, G7, G10, and G15, respectively) or G3 + 250 μmol/l tolbutamide (G3 + T). Four pulses of increasing concentrations of arginine were applied as indicated. [Ca\textsuperscript{2+}] recordings and insulin measurements started after 15 and 30 min of the equilibration period, respectively. Note that the time scale for insulin secretion is two times that for [Ca\textsuperscript{2+}]. Results are means ± SE for 11–12 islets from 3 preparations (A and C) and perifusions with islets from 6 distinct preparations (B and D).
min (pulses) or 10 vs. 20 min (stepwise increases). However, to compare the two parameters, average insulin secretion rate was computed over shorter periods: 6 min for pulses and 12 min for stepwise increases. These dose curves of arginine and tolbutamide effects were computed from individual experiments and averaged. In another series of experiments, only one concentration of arginine or tolbutamide was applied for 20 min, so that [Ca\(^{2+}\)]\(_i\) and insulin secretion could be measured over the same period of time. The statistical significance of differences between means was assessed by ANOVA followed by a Newman-Keuls test.

RESULTS

Glucose modulation of arginine effects. Mouse islets were perifused with 3, 7, 10, or 15 mmol/l glucose (G3, G7, G10, and G15, respectively) or with 3 mmol/l glucose + 250 μmol/l tolbutamide (G3 + T). In the first series of experiments, four pulses of increasing concentrations of arginine (1, 3, 5, and 10 mmol/l) were applied sequentially (Fig. 1). Before stimulation with arginine, both [Ca\(^{2+}\)]\(_i\) (Fig. 1, A and C) and insulin secretion (Fig. 1, B and D) increased with the concentration of glucose (\(P < 0.001\)). In G3, the pulses of arginine slightly increased basal [Ca\(^{2+}\)]\(_i\), but did not affect insulin secretion. The efficacy of arginine on both [Ca\(^{2+}\)]\(_i\) and insulin secretion was much greater in G7, G10, and G15 (Fig. 1). The combination G3 + T increased [Ca\(^{2+}\)]\(_i\), as much as did G15 (Fig. 1, A and C) but was much less efficient on insulin secretion (compare Fig. 1, B and D; note the different scales). However, tolbutamide unmasked clear effects of the arginine pulses on [Ca\(^{2+}\)]\(_i\) and insulin secretion (Fig. 1, A and B).

In the second series of experiments, the concentration of arginine was increased stepwise. This resulted in only minor increases in [Ca\(^{2+}\)]\(_i\), and did not stimulate insulin secretion in G3 (Fig. 2, A and B). At higher glucose concentrations, gradual increases in [Ca\(^{2+}\)]\(_i\) and insulin secretion followed each step of arginine application. Again, arginine produced concentration-dependent increases in [Ca\(^{2+}\)]\(_i\) and insulin secretion in G3 + T (Fig. 2, A and B).

The results obtained in both series were combined to compute the concentration-response curves shown in Fig. 3. Arginine caused a concentration-dependent increase in [Ca\(^{2+}\)]\(_i\), under all tested conditions. Figure 3A also shows that absolute [Ca\(^{2+}\)]\(_i\) reached in the presence of each arginine concentration increased with the glucose concentration. Because this absolute [Ca\(^{2+}\)]\(_i\) at least partly depends on the glucose concentration, changes (Δ) in [Ca\(^{2+}\)]\(_i\) produced by arginine were also calculated (Fig. 3C). The increment produced by each arginine concentration augmented (\(P < 0.01\) or less) between G3 and

Fig. 2. Effects of stepwise increases in arginine concentration on [Ca\(^{2+}\)]\(_i\) (A and C) and insulin secretion (B and D) in mouse islets. Islets were perifused throughout with G3–G15 or G3 + T, and the concentration of arginine was increased sequentially as indicated. Results are means ± SE for 10–12 islets from 3 preparations (A and C) and perifusions with islets from 6 distinct preparations (B and D).
G7 and between G7 and G10 (except at 1 mmol/l arginine), with no further difference between G10 and G15. Arginine also produced a concentration-dependent increase in insulin secretion except in G3. Both the absolute secretion rate (Fig. 3B) and the insulin secretion (Fig. 3D) evoked by each arginine concentration increased with the concentration of glucose (P < 0.001). These changes in insulin secretion were qualitatively similar but not proportional to the [Ca^{2+}] changes produced by arginine. For example, [Ca^{2+}] produced by 5 mmol/l arginine in G7 was similar to that produced by 3 mmol/l arginine (Arg3) in G10–G15 (Fig. 3C), but insulin secretion was only weakly stimulated in G7 + 5 mmol/l arginine, and much more so in G15 + 3 mmol/l arginine than in G10 + 3 mmol/l arginine (P < 0.001; Fig. 3D). Clearly, differences in insulin secretion were qualitatively similar but not proportional to the [Ca^{2+}] changes produced by arginine.

The dissociation between [Ca^{2+}] and insulin secretion rate was even more striking in the experiments performed in G3 + T. At all arginine concentrations, [Ca^{2+}] was higher (or similar; Fig. 3A), but insulin secretion was considerably lower (P < 0.001; Fig. 3B) in G3 + T than in G10.

The explanation lies in the amplifying action of glucose, as shown by the relationship between insulin secretion and [Ca^{2+}] in the presence of the different arginine concentrations (Fig. 4A). The linear relationship between insulin secretion and [Ca^{2+}] at increasing arginine concentrations was distinctly influenced by glucose and tolbutamide. Compared with G7, G3 + T caused a rightward shift, whereas G10 and G15 caused rightward and upward shifts of the relationship (Fig. 4A). The only effect of tolbutamide was thus to augment the rise in [Ca^{2+}] produced by arginine, whereas glucose augmented both the arginine-induced [Ca^{2+}] rise and the amount of insulin secreted at each [Ca^{2+}] level.

In the previous experiments, the same arginine concentrations were tested at different glucose concentrations, which resulted in different β-cell [Ca^{2+}] levels. In an additional series, performed three years after the first ones, we sought to achieve similar [Ca^{2+}] levels by applying different arginine concentrations at...
two conditions, although arginine under the three conditions (Fig. 5). Insulin secretion was induced by 10 mmol/l arginine in G7 (initial peak) or in G7-T5 (steady state; Fig. 5). Insulin secretion was induced by increasing arginine (3 mmol/l) increased [Ca\(^{2+}\)]i, without affecting insulin secretion, which was stimulated by the larger [Ca\(^{2+}\)], rises induced by T100 (Fig. 6, A and B) and T250 (data not shown). At higher glucose concentrations, both [Ca\(^{2+}\)], and insulin responses to tolbutamide increased with the concentration of the drug. In G15, they saturated at T10–25 (Fig. 6, C and D).

In a second series of experiments similar to that performed with arginine, tolbutamide was applied stepwise (data not shown). The results were essentially similar to those obtained with tolbutamide pulses. In G3, only T100 and T250 were effective. In G7 and G10, a concentration-dependent increase in [Ca\(^{2+}\)], and insulin secretion occurred between T5 and T100. In G15, both responses reached a maximum at T10–25.

Both series were used to compute the concentration-response curves shown in Fig. 7. At all glucose concentrations, tolbutamide produced a concentration-dependent increase in [Ca\(^{2+}\)], with a plateau above T25 in G7–G15 (Fig. 7A). At each tolbutamide concentration, absolute [Ca\(^{2+}\)], increased with the glucose concentration. However, above G3, this was largely the result of the differences in initial [Ca\(^{2+}\)], (in the absence of tolbutamide) because ∆[Ca\(^{2+}\)], produced by the drug was not glucose dependent in G7–G15 (Fig. 7C). The effects of tolbutamide on insulin secretion were also concentration and glucose dependent. The absolute rate of secretion increased with the glucose concentration at all tolbutamide concentrations (Fig. 7B), but the ∆insulin did not increase with glucose above G10 (Fig. 7D).

Although tolbutamide increased both [Ca\(^{2+}\)], and insulin secretion, both effects were not proportional when the comparison was made at different glucose concentrations. For example, T250 was a poor secretagogue in G3, although it increased [Ca\(^{2+}\)], to values at which G7 + T10 or G10 + T5 induced strong secretory responses (Fig. 7, A and B). Also striking is the difference in insulin secretion evoked by T10–T100 in G7 and G10 compared with the similarity of the changes in absolute or ∆[Ca\(^{2+}\)]. This discrepancy can again be explained by the amplifying action of glucose. Compared with G3, the linear relationship between insulin secretion and [Ca\(^{2+}\)], at increasing tolbutamide concentrations was shifted rightward and upward by glucose (Fig. 4B). This shows that, in addition to augmenting the effect of tolbutamide on [Ca\(^{2+}\)], glucose increases the amount of insulin secreted at each [Ca\(^{2+}\)].

In an independent series of experiments, we sought to achieve similar [Ca\(^{2+}\)], during application of tolbutamide at different glucose concentrations and measured [Ca\(^{2+}\)], and insulin secretion during the same periods of time. In G5, T5 barely affected [Ca\(^{2+}\)] and did not increase insulin secretion (Fig. 5, C and D). [Ca\(^{2+}\)] was similarly increased after addition of either T100 to G5 or T5 to G10, both during the initial

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**Fig. 4.** Correlations between insulin secretion and [Ca\(^{2+}\)], in islets stimulated by increasing arginine (A) or tolbutamide (B) concentrations. A: arginine experiments. Data are taken from Fig. 3, but for each concentration of arginine the values for pulses and plateaus are shown here separately, hence, 8 symbols at each glucose concentration. Separate regression lines were calculated for the experiments performed at each glucose concentration (G3–G15 and G3 + T), and the coefficient of correlation (R) and P value are given in the boxes. NS, not significant. B: tolbutamide experiments. Data are taken from Fig. 6 and presented in a similar way as those of the arginine experiments.
peak (however, with a 1-min temporal shift) and steady state. However, insulin secretion induced by T100 in G5 was significantly smaller than that induced by T5 in G10 except at two early time points.

DISCUSSION

The amplifying action of glucose on insulin secretion is defined as the increase in insulin secretion that the sugar produces without causing a further increase in an already elevated β-cell \([\text{Ca}^{2+}] \text{i}\) (26). Preventing glucose from changing steady-state \([\text{Ca}^{2+}] \text{i}\) is possible with maximally effective concentrations of sulfonylureas, but not with arginine alone. Moreover, this approach would be inappropriate in the present study, the aim of which was to establish whether glucose potentiation of arginine- or tolbutamide-induced insulin secretion involves increases in the triggering signal (larger rise in \([\text{Ca}^{2+}] \text{i}\)), amplification of the secretory response to that \([\text{Ca}^{2+}] \text{i}\) rise, or both. By no means did we attempt to identify the molecular mechanisms implicated in this amplification. We therefore compared the influence of glucose on the magnitude of \([\text{Ca}^{2+}] \text{i}\), and insulin secretion changes induced by arginine and tolbutamide.

We first show that the arginine-induced \([\text{Ca}^{2+}] \text{i}\) rise is concentration dependent and that the effect of each arginine concentration on \([\text{Ca}^{2+}] \text{i}\) increases with the ambient glucose concentration. The mechanism by which glucose augments arginine-induced \([\text{Ca}^{2+}] \text{i}\) rise can be explained as follows. The transport of cationic arginine into β-cells (8) produces an inward current (44), the depolarizing action of which increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane. Because this resistance mainly depends on the closure of \(K_{\text{ATP}}\) channels, the depolarizing action of arginine increases with the electrical resistance of the plasma membrane.

Fig. 5. Effects of arginine (Arg; 3 or 10 mmol/l) and tolbutamide (Tolb; 5 or 100 mmol/l) on \([\text{Ca}^{2+}] \text{i}\), (A and C) and insulin secretion (B and D) in mouse islets. Islets were perifused throughout with the indicated glucose concentration. Results are means ± SE for 15 islets from 3 preparations (A and C) and perifusions with islets from 6–9 distinct preparations (B and D).
ability of arginine to depolarize β-cells (28) and raise [Ca^{2+}]_{i} (this study), even in low glucose.

We next confirm that, in vitro, arginine increases insulin secretion in a concentration-dependent manner and that the effect of a given concentration of arginine augments with the glucose concentration (14, 36, 37). On the basis of our [Ca^{2+}]_{i} measurements, the simplest explanation for the interaction between glucose and arginine would be that glucose augments the rise in [Ca^{2+}]_{i}, produced by arginine. However, the present study clearly shows that this increase in the triggering signal is not a sufficient explanation. At a fixed glucose concentration of 7 mmol/l or higher, arginine-induced insulin secretion was proportional to [Ca^{2+}]_{i} in islets. However, this relationship changed with the glucose concentration. When, at different glucose concentrations, [Ca^{2+}]_{i} was increased to similar values by different arginine concentrations, insulin secretion was not equivalent but increased with glucose. This phenomenon, characterized by a greater efficacy of Ca^{2+} on secretion, corresponds to the amplifying action of glucose (26). Its importance is underlined by the experiments combining tolbutamide and arginine. The sulfonylurea mimicked the ability of glucose to augment the arginine-induced [Ca^{2+}]_{i} rise, but the impact on insulin secretion was considerably less than that of the sugar.

Our findings explain how tolbutamide potentiates arginine-induced insulin secretion in vivo (39, 40) and why the potentiating action of the sulfonylurea is smaller than that of glucose unless the sugar is infused simultaneously to prevent any fall in blood glucose (39).

While the mechanisms of β-cell stimulation by arginine and glucose differ markedly, those of the stimulation by tolbutamide and glucose overlap at least partly; K_{ATP} channels are their common target. Closure of an increasing number of K_{ATP} channels is an essential event in the concentration-dependent depolarization and subsequent increase in electrical activity and [Ca^{2+}]_{i} (3, 9, 24, 29) that both agents produce. The direct blockade of the channels by tolbutamide through interaction with sulfonylurea receptor 1 and the indirect blockade by glucose via changes in metabolism are relatively well understood, but the interaction of the two agents is complex (1). Whatever the molecular mechanisms of this interaction, the relevant functional result is, as shown in the present study, that an increase in the glucose concentration augments the islet [Ca^{2+}]_{i} rise produced by tolbutamide.

Glucose potentiation of tolbutamide-induced insulin secretion has been well characterized in vitro (34) and in vivo (7). Our results show that one important underlying mechanism is...
an increase in the triggering signal, the \([\text{Ca}^{2+}]_i\) rise, produced by the sulfonylurea. We also establish that this explanation is incomplete. Thus, for an eventually similar \([\text{Ca}^{2+}]_i\) in islets, insulin secretion augments with the glucose concentration, which demonstrates that amplification of the action of \([\text{Ca}^{2+}]_i\) on exocytosis (26) also markedly contributes to this potentiation of tolbutamide-induced insulin secretion by glucose. Interestingly, the potentiation of the \([\text{Ca}^{2+}]_i\) rise occurred between 3 and 7 mmol/l glucose, not above, and was observed in the presence of therapeutic, not high, concentrations of tolbutamide. This is not surprising if one considers that glucose and tolbutamide produce the triggering signal by acting on the same target, K\(_\text{ATP}\) channels. In contrast, the amplification of insulin secretion extended to 10–15 mmol/l glucose and persisted at high concentrations of tolbutamide. This is not surprising if one considers that glucose and tolbutamide produce the triggering signal by acting on the same target, K\(_\text{ATP}\) channels. In contrast, the amplification of insulin secretion extended to 10–15 mmol/l glucose and persisted at high concentrations of tolbutamide.

In conclusion, glucose potentiation of arginine- or tolbutamide-induced insulin secretion involves the following two mechanisms: an increase in the ability of the amino acid or the sulfonylurea to augment \([\text{Ca}^{2+}]_i\), the triggering signal, and an amplification of the efficacy of this signal on exocytosis. While the potentiation of the \([\text{Ca}^{2+}]_i\) rise is relatively well understood mechanistically, the amplifying mechanism is not. There are no reasons to postulate that it is different from the K\(_\text{ATP}\) channel-independent pathway by which glucose amplifies its own effects on insulin secretion (26, 30), possibly by augmenting the number of granules available for exocytosis (45). It is well established that the amplifying pathway requires glucose metabolism (26, 42), in particular an increased flux through the tricarboxylic acid cycle (33), whereas activation of protein kinases A and C (30, 42) is not involved, which clearly distinguishes this metabolic amplification from the potentiation by neurohormones. A number of potential signals, like glutamate, have been excluded (5, 33). Possible candidates currently include adenine and guanine nucleotides (42), changes in \(\text{K}_i\) or granular pH (4), short-lived proteins (13), and an increase in long-chain acyl-CoAs with subsequent acylation of various proteins (10, 46, 51). Whatever the underlying molecular mechanisms, the dual action of glucose on the magnitude and efficacy of the \(\text{Ca}^{2+}\) signal constitutes a protection mechanism by which moderate hypoglycemia reduces the insulin secretory response to sulfonylureas (2). It must also be taken into consideration for the interpretation of the alterations of the potentiating action of glucose in type 2 diabetic patients. Finally, our data should help with understanding why arginine-induced responses, not only glucose-induced re-

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**Fig. 7.** Influence of the tolbutamide concentration on \([\text{Ca}^{2+}]_i\) (A and C) and insulin secretion (B and D) in mouse islets. Data were computed from the experiments shown in Fig. 6 and another, not shown, series in which tolbutamide was applied in plateaus of increasing concentrations. Absolute \([\text{Ca}^{2+}]_i\) and insulin secretion rates are shown in A and B, respectively. \(\Delta[\text{Ca}^{2+}]_i\) and Ainsulin secretion above prestimulatory values are shown in C and D, respectively. Values are means ± SE (when larger than the symbol size) for 22–24 islets (A and C) and 12–13 perfusions (B and D).
 sponses, are altered in certain animal models (31) or in humans (27) with a genetically identified cause of islet dysfunction.

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REFERENCES


