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Sulfonylurea Mimics the Effect of Glucose in Inducing Large Amplitude Oscillations of Cytoplasmic Ca^{2+} in Pancreatic β-Cells

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SUMMARY

The effects of the insulin-releasing sulfonylurea tolbutamide on the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) in individual pancreatic β-cells or suspensions of β-cells were analyzed using the probe fura-2 and dual-wavelength fluorometry. Subsequent additions of 1, 10, and 100 μM tolbutamide induced a graded response, ranging from a single $[Ca^{2+}]_i$ peak to a sustained increase. These effects depended on the presence of extracellular Ca^{2+} and were reversed by the hyperglycemic sulfonamide diazoxide. The responses were diminished in the presence of albumin and varied considerably between different cells. Sometimes tolbutamide triggered slow large amplitude oscillations in $[Ca^{2+}]_i$ similar to those induced by glucose. The increase in $[Ca^{2+}]_i$ during each tolbutamide-induced oscillation was often more rapid than for glucose-induced oscillations. Oscillations or steady state increases in $[Ca^{2+}]_i$ induced by glucose were little influenced by tolbutamide. However, subthreshold concentrations of glucose could reactivate $[Ca^{2+}]_i$ response to tolbutamide that had declined. Although in several ways the abilities of glucose and tolbutamide to raise $[Ca^{2+}]_i$ were similar, the sulfonylurea lacked a $[Ca^{2+}]_i$-lowering component. The latter effect of glucose was so pronounced that an increase of its concentration from 3 to 20 mM caused temporary lowering of $[Ca^{2+}]_i$ to the basal level, even during tolbutamide stimulation. The results indicate that closure of the ATP-sensitive K^+-channels is important for the large amplitude oscillations of $[Ca^{2+}]_i$, the appearance of which reflects the balance between entry of Ca^{2+} through the voltage-dependent channels and its removal from the cytoplasm.

The mechanism by which hypoglycemic sulfonylureas initiate the increase in the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) triggering insulin release has been the subject of considerable debate (1). With the patch-clamp technique, it became possible to demonstrate that these drugs depolarize the β-cells by blocking the same ATP-sensitive K^+ channels in the β-cell membrane that are closed by glucose (2, 3). It is now believed that depolarization by this mechanism with resulting influx of Ca^{2+} through the voltage-dependent channels is the major explanation for stimulation of secretion by sulfonylureas (4, 5). Studies of islet cell (6, 7) and clonal β-cell (8) suspensions have confirmed that sulfonylureas increase $[Ca^{2+}]_i$.

The fact that glucose and sulfonylureas depolarize the β-cells through a common route does not imply that their actions on $[Ca^{2+}]_i$ are identical. Apart from triggering Ca^{2+} influx, there is also a $[Ca^{2+}]_i$-lowering component in the action of glucose (9–12), which can be attributed to stimulation of Ca^{2+} sequestration and outward transport.

Glucose concentrations stimulating insulin release submaximally induce a characteristic burst pattern of rhythmic depolarization of β-cells in intact pancreatic islets (4, 13). It was predicted that $[Ca^{2+}]_i$ oscillates in phase with the bursts of depolarization due to the alternating dominance of Ca^{2+} influx through the voltage-dependent channels and removal of the cation by sequestration and outward transport (14–16). The existence of glucose-induced oscillations of $[Ca^{2+}]_i$ was indeed verified by measurements on individual β-cells (12, 17, 18). However, each oscillation had a duration of 2–6 min, which is considerably longer than a typical burst of membrane depolarization lasting for 10–40 sec. The reason for this discrepancy is still unclear.

In an attempt to elucidate the mechanisms for regulation of $[Ca^{2+}]_i$ in the pancreatic β-cells, we have now taken advantage of the facts that glucose and tolbutamide depolarize the β-cells by closing the same K^+ channels and that the two agents have different effects on Ca^{2+} removal from the cytoplasm. It will be demonstrated that tolbutamide also can induce large amplitude oscillations of $[Ca^{2+}]_i$. The results support the concept that the large amplitude oscillations of $[Ca^{2+}]_i$ and bursts of membrane depolarization are different reflections of a single phenomenon.

Experimental Procedures

Materials. Reagents of analytical grade and deionized water were used. Collagenase and HEPES were obtained from Boehringer Mann-

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ABBREVIATIONS: HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; EGTA, [ethyleneglycol-bis(oxyethylenenitrilo)]tetraacetic acid.

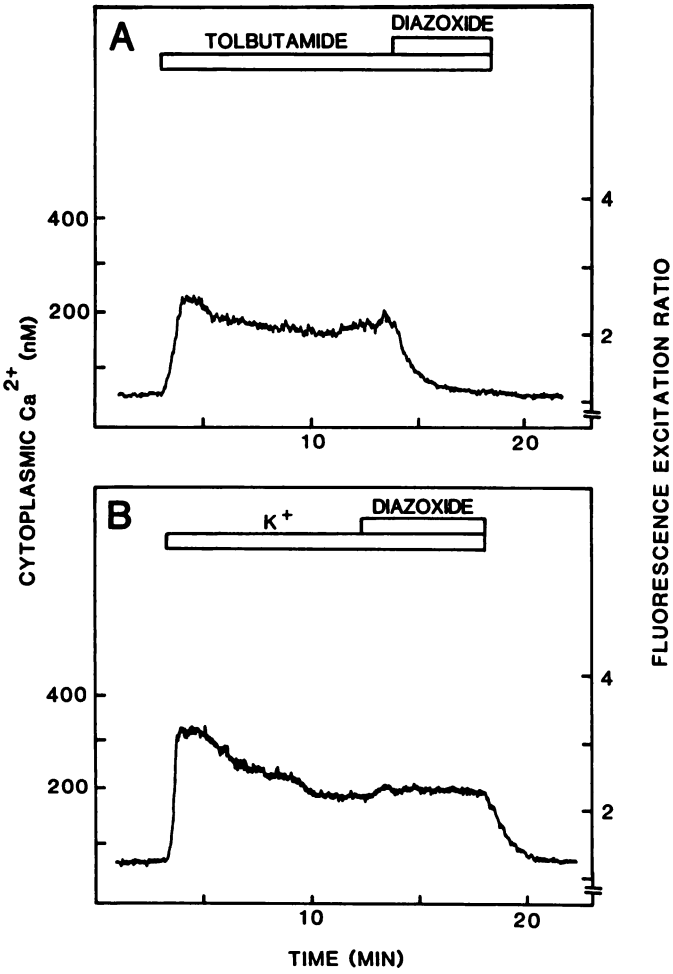

Fig. 1. Effects of tolbutamide, K^+ depolarization and diazoxide on the cytoplasmic Ca^{2+} of individual β-cells exposed to 3 mM glucose in an albumin-free medium. The 340/380 nm fluorescence excitation ratios of the indicator fura-2 are shown on the *right* and the corresponding $[Ca^{2+}]_i$ values (nM) on the *left*. After loading with fura-2, the cells were superfused with an albumin-free medium containing 3 mM glucose. During the periods indicated by *horizontal bars*, 100 μM tolbutamide and 400 μM diazoxide were added or the K^+ concentration was raised from 5.9 to 30.9 mM.

heim GmbH (Mannheim, FRG); bovine serum albumin (fraction V), EGTA, Tris, and Triton X-100 from Sigma; tolbutamide from Hoechst AG (Frankfurt/M, FRG); methoxyverapamil from Knoll AG (Ludwigshafen, FRG); and diazoxide from Schering (Kenilworth, NJ). Diethylenetriaminepentaacetic acid was from Merck (Darmstadt, FRG); glucose from BDH Chemicals (Poole, England); and the tetrapotassium salt of fura-2 and its acetoxymethylester from Molecular Probes Inc. (Eugene, OR).

Preparation of β-cells. Adult obese-hyperglycemic mice (gene symbol *ob/ob*) were from a local colony (19). The animals were killed by decapitation and pancreatic islets were isolated by a collagenase technique. Previous studies indicated that these islets contain more than 90% β-cells (19), which respond normally to glucose and other regulators of insulin release (20). The islets were dispersed into single cells by shaking in a Ca^{2+}-deficient medium, and β-cells were separated from debris by centrifugation through a medium containing Ca^{2+} and supplemented with 40 mg/ml bovine serum albumin (21). After suspension in RPMI 1640 culture medium supplemented with 10% fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10 μg/ml gentamicin, either the cells were allowed to attach to circular 25-mm cover glasses during 1–3 days in an incubator at 37°, with an atmosphere of 5% CO_2 in humidified air, or the suspensions were gently shaken for 1–3 hr in the incubator. The viability of the cells in suspension exceeded 85% and was close to 100% for those attached to cover glasses, as judged by trypan blue exclusion.

Loading with the fura-2 indicator. The subsequent experimental handling was performed with a basal medium physiologically balanced in cations, with Cl^- as the sole anion (22). When not otherwise stated, the medium used for loading with fura-2 was supplemented with 0.1 mg/ml bovine serum albumin and contained 3 mM glucose and 1.28 mM Ca^{2+}. In each experiment, 2–6 × 10^6 cells, representing the yield from one mouse, were washed and suspended in 5 ml of medium. Alternatively, the cover glasses with attached cells were rinsed and placed in Petri dishes containing 2 ml of medium. After addition of 0.5–1.0 μl/ml of 1 mM fura-2 acetoxymethylester in dimethyl sulfoxide, the cells were incubated at 37° for 40 min. The cells in suspension or those attached to cover glasses were then washed and incubated or superfused with a medium containing 1.28 mM Ca^{2+}, 3 mM glucose, and 0.1% albumin, unless otherwise stated in the legends to the figures.

Measurements of cytoplasmic Ca^{2+} in cell suspensions. The rinsed and pelleted cells were resuspended in 1 ml of the rinsing medium and transferred to a 1-cm quartz cuvette placed in the thermostatically controlled (37°) cuvette holder of a time-sharing multichannel spectrophotofluorometer (23), set for continuous recording of the 340/380 nm fluoresence excitation ratio. The dynamic response of this equipment exceeded by at least 2 orders of magnitude the most rapid effects observed here. Further information about the system and its properties have been described previously (9). There was some leakage of the fura-2 indicator from the cells. The loss of intracellular fura-2 is no problem with the dual-wavelength approach (9), but the contribution of interfering extracellular indicator to the fluorescence ratio signal had to be estimated. This was accomplished by quenching the extracellular fura-2 fluorescence by adding 100 μM Ce^{3+} early in the experiments, followed by chelation of the cation by 150 μM diethylenetriaminepentaacetic acid (9). Leakage was unfortunately enhanced by glucose, and this increase was difficult to estimate in the individual experiments (9). The observation periods after addition of glucose were, therefore, limited to 5–7 min, assuming the same rate of leakage as before.

At the end of each experiment, the cells were disrupted by the addition of 0.05% Triton X-100. The 340/380 nm fluorescence excitation ratio and the 380 nm excitation fluorescence were then obtained both at saturating Ca^{2+} concentrations and at <1 nM Ca^{2+}. Reduction to the latter level was accomplished by the addition of excess EGTA as well as Tris to raise the pH above 8.3. $[Ca^{2+}]_i$ could now be calculated using the following equation (24):

$$[Ca^{2+}]_i = K_D\beta\,(R - R_{min})/(R_{max} - R)$$

where $\beta = F_o/F_s$. F_o and R_{min} are the fura-2 fluorescence at 380 nm and the 340/380 nm fluorescence excitation ratio, respectively, in medium lacking Ca^{2+}. F_s and R_{max} are the corresponding data obtained at saturating Ca^{2+} concentrations. The K_D for the Ca^{2+}-fura-2 complex employed was 231 nM (25).

Measurements of cytoplasmic Ca^{2+} in individual cells. The cover glasses with the loaded cells were used as the bottom of an open chamber designed for microscopic work (26). The chamber wall was a broad silicon rubber ring (9-mm inner diameter) pressed to the cover glass by the threaded chamber mount and a thin stainless steel ring. Cannulas fixed to this ring were connected to a two-channel peristaltic pump, allowing steady superfusion of a 2.5-mm medium layer at a rate of 1.0 ml/min. The chamber was placed on the stage of an inverted microscope (Leitz Diavert) within a climate box maintained at 37°. The microscope was equipped for epifluorescence fluorometry with a 100× UV-fluorite objective (Nikon). The equipment for continuously recording the 340/380 nm fluorescence excitation ratio was identical to that used for measurements in cell suspensions. Calibration was made according to the equation given above, but in this case the F_o, R_{min}, F_s, and R_{max} values were obtained in model experiments with drops of "intracellular" K^+-rich medium containing fura-2. Leakage of

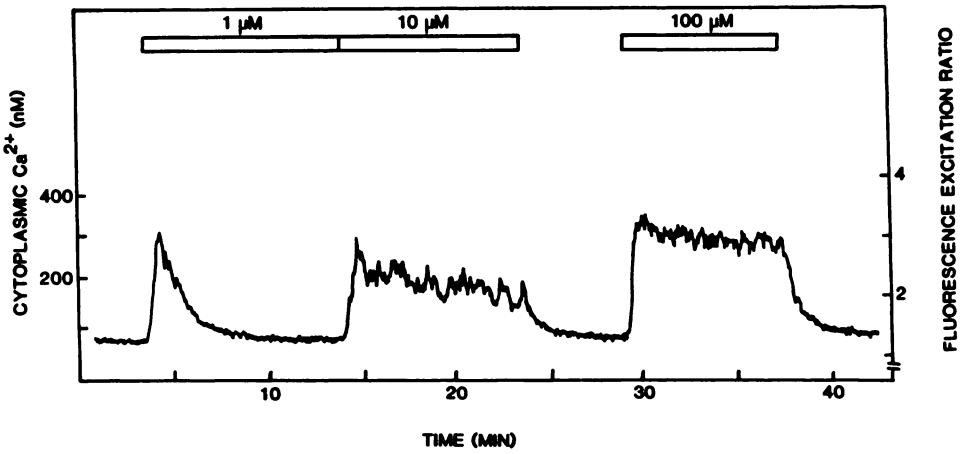

Fig. 2. Effects of increasing concentrations of tolbutamide on the cytoplasmic Ca^{2+} of an individual β-cell exposed to 3 mM glucose in an albumin-free medium. The 340/380 nm fluorescence excitation ratios of the indicator fura-2 are shown on the *right* and the corresponding $[Ca^{2+}]_i$ values (nM) on the *left*. After loading with fura-2, the cells were superfused with an albumin-free medium containing 3 mM glucose. During the periods indicated by the *horizontal bars*, 1, 10, or 100 μM tolbutamide was added.

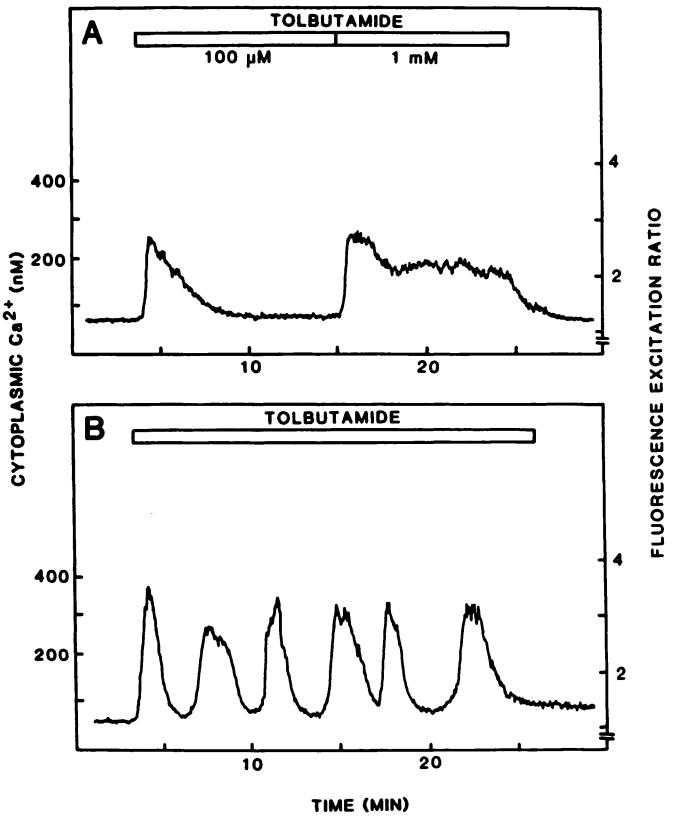

Fig. 3. Effects of tolbutamide on the cytoplasmic Ca^{2+} of individual β-cells exposed to 3 mM glucose in an albumin-containing medium. The 340/380 nm fluorescence excitation ratios of the indicator fura-2 are shown on the *right* and the corresponding $[Ca^{2+}]_i$ values (nM) on the *left*. After loading with fura-2, the cells were superfused with a medium containing 3 mM glucose and 0.1 mg/ml albumin. During the periods indicated by the *horizontal bars*, 100 μM (A and B) or 1 mM (A) tolbutamide was added.

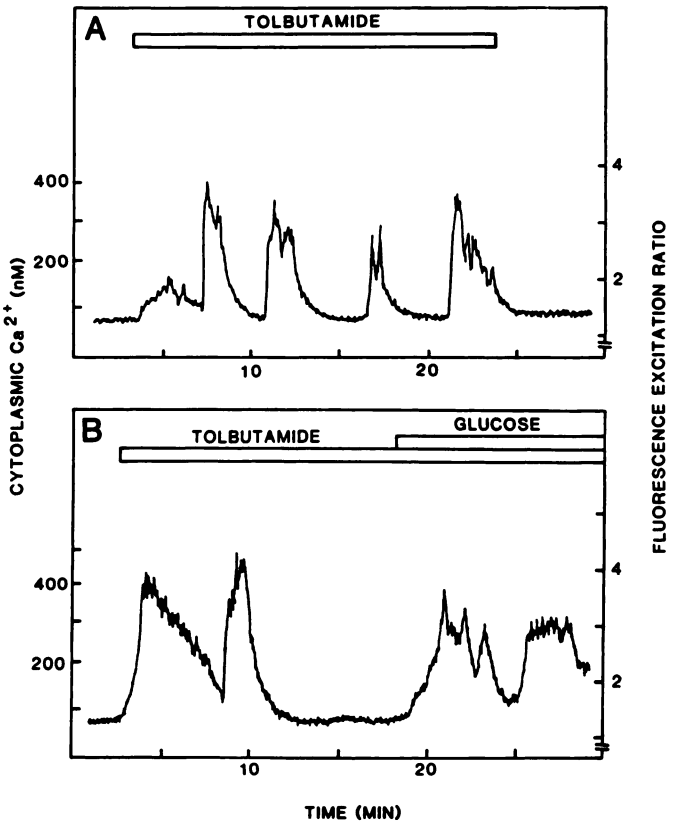

Fig. 4. Effects of tolbutamide on the cytoplasmic Ca^{2+} of individual β-cells exposed to an albumin-containing medium lacking glucose. The 340/380 nm fluorescence excitation ratios of the indicator fura-2 are shown on the *right* and the corresponding $[Ca^{2+}]_i$ values (nM) on the *left*. After loading with fura-2 in the absence of glucose, the cells were superfused with a glucose-free medium containing 0.1 mg/ml albumin. During the periods indicated by the *horizontal bars*, 100 μM (A) or 1 mM (B) tolbutamide was added, as well as 3 mM glucose (B).

indicator represented no problem with the superfusion approach, because extracellular indicator is rapidly diluted and removed.

Statistical analyses. Results are presented as means ± standard errors. Statistical analyses were by Student's *t* test for paired data.

Results

When 100 μM tolbutamide was introduced to individual β-cells superfused with an albumin-free medium containing 3 mM glucose, there was a prompt increase in $[Ca^{2+}]_i$ which was reversed upon subsequent addition of 400 μM diazoxide (Fig. 1A). Depolarization with excessive K^+ caused a similar rapid rise in $[Ca^{2+}]_i$, but this action was unaffected by diazoxide (Fig. 1B).

After different concentrations of tolbutamide were tested under the same experimental conditions, it was obvious that the response was graded. In some cells it was possible to obtain a transient peak of $[Ca^{2+}]_i$ upon exposure to as little as 1 μM tolbutamide (Fig. 2). A 10-fold higher concentration gave a sustained increase, which was less pronounced than that subsequently obtained with 100 μM drug. When the medium con-

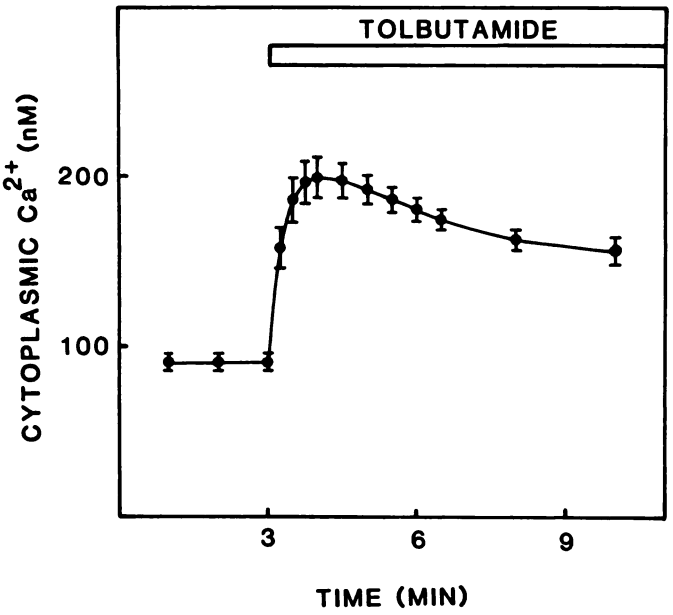

Fig. 5. Average effect of tolbutamide on the cytoplasmic Ca^{2+} of β-cells in suspension exposed to 3 mM glucose in an albumin-containing medium. The 340/380 nm fluorescence excitation ratios of the indicator fura-2 were used to calculate the $[Ca^{2+}]_i$ values (nM) shown on the *left*. After loading with fura-2, the cells were rinsed and incubated in a medium containing 3 mM glucose and 0.1 mg/ml albumin. Tolbutamide (100 μM) was added, as indicated by the *horizontal bar*.

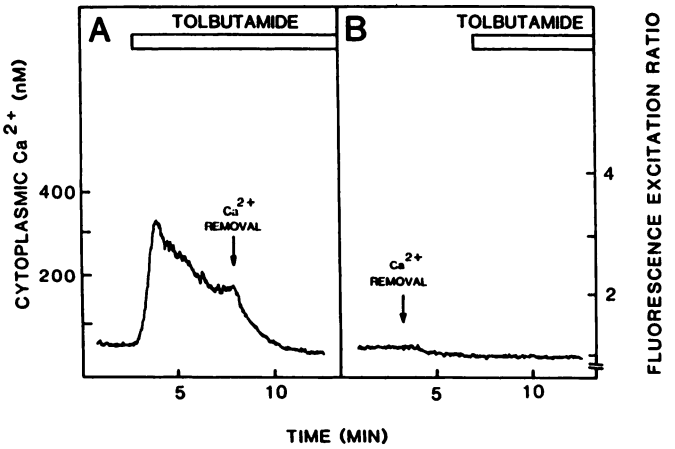

Fig. 6. Effects of Ca^{2+} removal on the actions of tolbutamide on the cytoplasmic Ca^{2+} of individual β-cells exposed to 3 mM glucose in an albumin-containing medium. The 340/380 nm fluorescence excitation ratios of the indicator fura-2 are shown on the *right* and the corresponding $[Ca^{2+}]_i$ values (nM) on the *left*. After loading with fura-2, the cells were superfused with a medium containing 3 mM glucose and 0.1 mg/ml albumin. During the periods indicated by the *horizontal bars*, 1 mM tolbutamide was added. Ca^{2+} was removed and 0.5 mM EGTA added as indicated by the *arrows*.

taining 3 mM glucose was supplemented with 0.1 mg/ml albumin, tolbutamide became less potent. In 18 experiments, addition of 100 μM sulfonylurea, thus, caused a sustained increase in 6 (e.g., Fig. 9) and a transient peak in 8 (Fig. 3A) cells. Even 1 mM tolbutamide gave a transient in 2 and a sustained increase in $[Ca^{2+}]_i$ in 2 (Fig. 3A) of 4 experiments. Two or more large amplitude oscillations similar to those induced by glucose (cf. Fig. 7) were observed in 4 of the 18 cells exposed to 100 μM tolbutamide in the presence of 3 mM glucose (Fig. 3B). These oscillations were characterized by nadirs close to basal $[Ca^{2+}]_i$

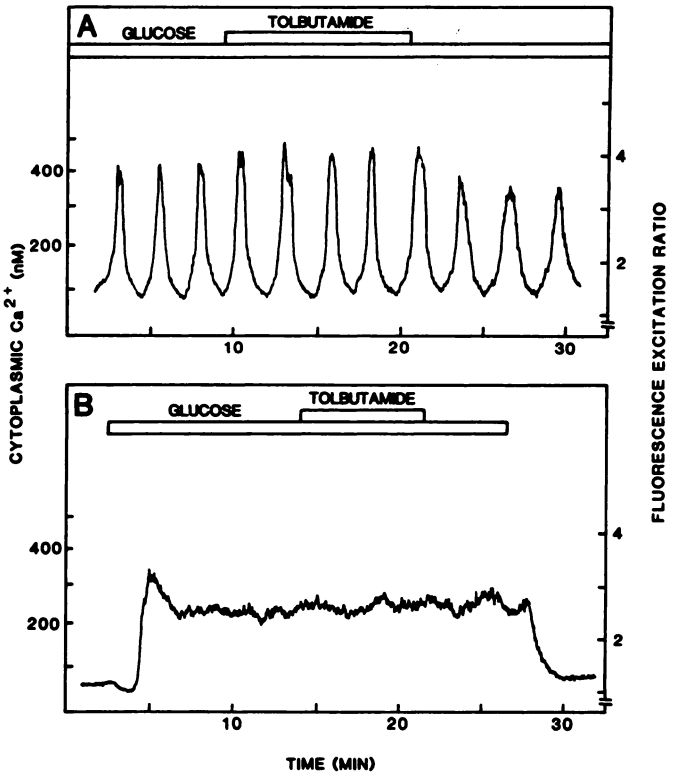

Fig. 7. Effects of tolbutamide on the cytoplasmic Ca^{2+} of individual β-cells exposed to 11 or 20 mM glucose in an albumin-containing medium. The 340/380 nm fluorescence excitation ratios of the indicator fura-2 are shown on the *right* and the corresponding $[Ca^{2+}]_i$ values (nM) on the *left*. After loading with fura-2, the cells were superfused with a medium containing 3 mM glucose and 0.1 mg/ml albumin. During the periods indicated by the *horizontal bars*, the glucose concentration was raised to 11 mM (A) or 20 mM (B) and 100 μM tolbutamide was added.

and peaks severalfold higher. In 26 experiments performed in the absence of glucose, 100 μM tolbutamide induced a single $[Ca^{2+}]_i$ peak in 12 cells, two or more oscillations in 7 cells (Fig. 4A), and an elevated $[Ca^{2+}]_i$ in another 7 cells. The corresponding figures in 14 experiments with 1 mM tolbutamide were 3, 3, and 8 cells with single peaks, two or more large amplitude oscillations (Fig. 4B), and elevated levels, respectively. Abrupt onsets of the $[Ca^{2+}]_i$ peaks, evident in Fig. 4, were observed in 7 of 10 cells oscillating in glucose-free medium containing tolbutamide and in 1 of 4 cells oscillating after exposure to the sulfonylurea in the presence of 3 mM glucose. The action of low glucose concentrations on $[Ca^{2+}]_i$ was additive to that of tolbutamide, as indicated from the immediate reappearance of peaks or oscillations when the sugar concentration was raised from 0 to 3 mM in three experiments (Fig. 4B). Fig. 5 shows the average effect of 100 μM tolbutamide on β-cells suspended in a medium containing 0.1 mg/ml albumin and 3 mM glucose. It is evident that an initial broad peak is followed by a sustained increase at a somewhat lower level.

The action of tolbutamide on the pancreatic β-cells depended strictly on influx of external Ca^{2+}. Removal of Ca^{2+} (Fig. 6A) or addition of 50 μM levels of the Ca^{2+} channel-blocker methoxyverapamil (not shown), thus, promptly reversed the sustained increase of $[Ca^{2+}]_i$ observed in cells exposed to 1 mM levels of the sulfonylurea. In the absence of Ca^{2+} (Fig. 6B) or presence of methoxyverapamil (not shown), introduction of the drug had no effect.

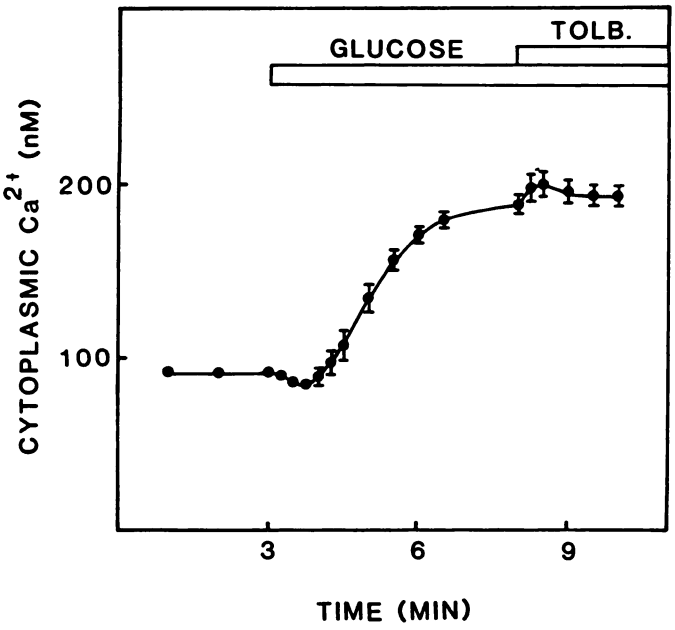

Fig. 8. Average effect of tolbutamide on the cytoplasmic Ca^{2+} of β-cells in suspension after raising glucose from 3 to 20 mM in an albumin-containing medium. The 340/380 nm fluorescence excitation ratios of the indicator fura-2 were used to calculate the $[Ca^{2+}]_i$ values (nM) shown on the *left*. After loading with fura-2, the cells were incubated in a medium containing 3 mM glucose and 0.1 mg/ml albumin. The glucose concentration was raised to 20 mM and 100 μM tolbutamide (*TOLB.*) was added, as indicated by the *horizontal bars*.

When 100 μM tolbutamide was added at 11 mM glucose, the sugar-induced large amplitude oscillations of $[Ca^{2+}]_i$ were marginally enhanced (Fig. 7A). An increase of the sugar concentration from 3 to 20 mM caused initial lowering of $[Ca^{2+}]_i$, followed by an increase that was often sustained (Fig. 7B). Addition of 100 μM tolbutamide had no effect on this elevated level. Fig. 8 shows the average effect of such an increase of the glucose concentration followed by addition of tolbutamide. Also, in this case, the action of the sulfonylurea was very modest.

When the glucose concentration was raised from 3 to 20 mM during stimulation with 100 μM tolbutamide, there was a temporary decrease in $[Ca^{2+}]_i$ to the basal level, followed after about 90 sec by return to a sustained increase (Fig. 9). The average effect of raising the glucose concentration from 3 to 20 mM on $[Ca^{2+}]_i$ of suspended β-cells during stimulation with 100 μM tolbutamide is shown in Fig. 10. Also, in this case, the increase in the sugar concentration resulted in a major temporary lowering of $[Ca^{2+}]_i$.

Discussion

Subpopulations of β-cells with different glucose sensitivities have been identified on the basis of changes in NAD(P)H and FAD fluorescence using fluorescence-activated cell sorting (27). A considerable variation in $[Ca^{2+}]_i$ responses is indeed a striking phenomenon in investigations of how a certain stimulus affects individual β-cells (18, 28). Studies of clonal β-cells have indicated that a particular cell reacts similarly to repeated stimulation, and this pattern has even been termed a Ca^{2+} fingerprint (28). The nature of individual cell responses is very important for understanding the mechanisms involved. However, measurements on many individual cells are required for general conclusions about their quantitative importance. In the present investigation, measurements on several individual β-cells in various experimental situations were done in parallel with analyses on suspensions of cells to assess average effects.

It is noteworthy that the $[Ca^{2+}]_i$ values calculated from the fura-2 fluorescence data are estimates based on the questionable assumptions that the Ca^{2+} concentration is homogeneous within the cytoplasm and that the indicator is restricted to this compartment. It is indeed likely that the variations of the Ca^{2+} concentration in certain parts of the cytoplasm are considerably more pronounced than is indicated from the measurements, due to restricted Ca^{2+} diffusion and the nonlinear characteristics of the fura-2 indicator (9).

Although the sensitivity to tolbutamide varied considerably between different cells, the cells investigated exhibited graded responses to increasing concentrations of the drug. The presence of albumin in the medium caused an apparent rightward shift of the dose-response relationship for the tolbutamide-induced rise in $[Ca^{2+}]_i$. This action was not unexpected, because albumin displaces sulfonylureas from the β-cell surface and lowers their potencies as insulin secretagogues (1, 29, 30).

The similarities between the $[Ca^{2+}]_i$-increasing actions of sulfonylureas and glucose were apparent from a number of experiments. As in previous studies with glucose (31), the hyperglycemic sulfonamide diazoxide, which activates the K^+

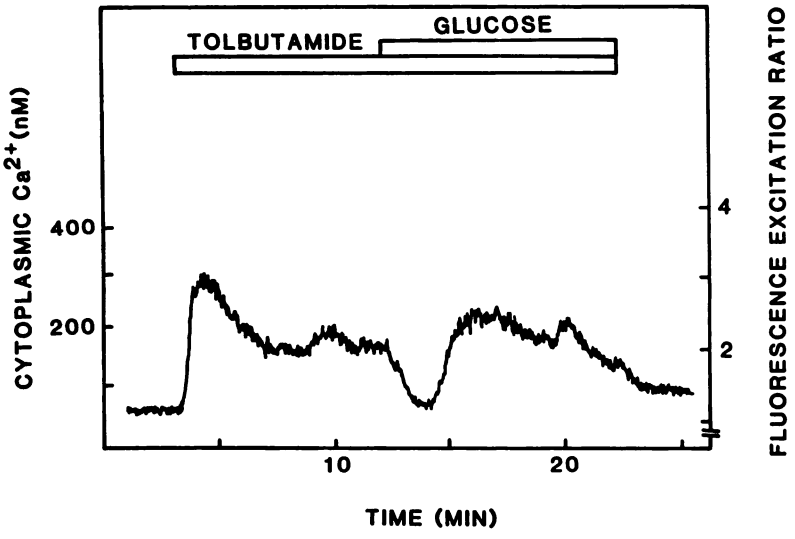

Fig. 9. Effects of raising glucose from 3 to 20 mM on the cytoplasmic Ca^{2+} of an individual β-cell stimulated by tolbutamide in an albumin-containing medium. The 340/380 nm fluorescence excitation ratios of the indicator fura-2 are shown on the *right* and the corresponding $[Ca^{2+}]_i$ values (nM) on the *left*. After loading with fura-2, the cells were superfused with a medium containing 3 mM glucose and 0.1 mg/ml albumin. During the periods indicated by the *horizontal bars*, 100 μM tolbutamide was added and the glucose concentration was raised to 20 mM.

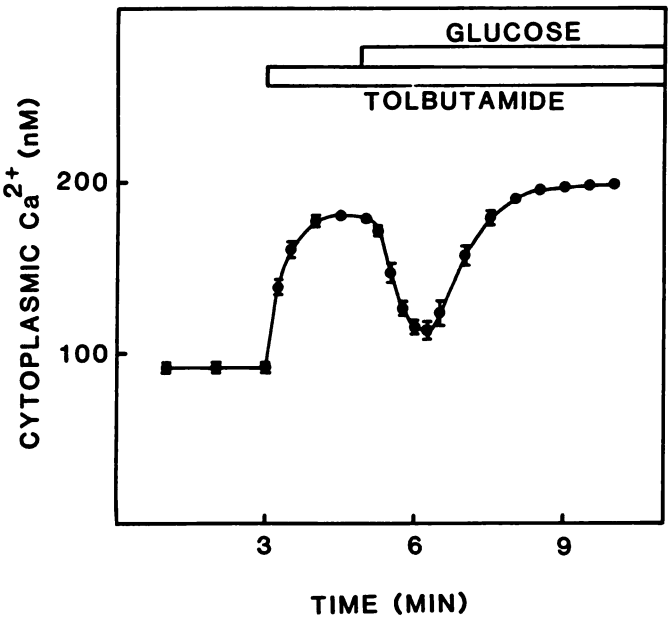

Fig. 10. Average effect of raising glucose from 3 to 20 mM on the cytoplasmic Ca^{2+} of β-cells in suspension stimulated by tolbutamide in an albumin-containing medium. The 340/380 nm fluorescence excitation ratios of the indicator fura-2 were used to calculate the $[Ca^{2+}]_i$ values (nM) shown on the *left*. After loading with fura-2, the cells were incubated in a medium containing 3 mM glucose and 0.1 mg/ml albumin. Tolbutamide (100 μM) was added and the glucose concentration was raised to 20 mM, as indicated by the *horizontal bars*.

channels blocked by glucose and sulfonylureas (3), reversed the tolbutamide-induced increase in $[Ca^{2+}]_i$. The specificity of this action was apparent from the fact that diazoxide did not influence the rise in $[Ca^{2+}]_i$ obtained with K^+ depolarization. As in the case of glucose, the tolbutamide-triggered increase in $[Ca^{2+}]_i$ depended entirely on the influx of Ca^{2+}. Moreover, the $[Ca^{2+}]_i$-increasing effect of tolbutamide was not or only marginally additive to that of high concentrations of glucose, whereas additive effects were obtained at low sugar concentrations. These data reinforce previous arguments (1) against the idea that sulfonylureas act as Ca^{2+} ionophores. The reason for the discrepancy between the frequency of glucose-induced $[Ca^{2+}]_i$ oscillations with periods of 2–6 min and bursts of membrane depolarization lasting for 10–40 sec (4, 13) is unclear. The slow oscillations of $[Ca^{2+}]_i$ are not specific for *ob/ob*-mice, inasmuch as they are also found in rat β-cells (18). It is possible that each oscillation corresponds to cyclic variations in the fraction of time spent at plateau potential during several consecutive burst cycles rather than to one individual burst (18). However, the variations of the burst cycles described so far (32, 33) are not sufficiently pronounced to explain the amplitude of the $[Ca^{2+}]_i$ oscillations, with minima often corresponding to basal $[Ca^{2+}]_i$. Moreover, because the time resolution of the measurements of $[Ca^{2+}]_i$ is about 10 msec, $[Ca^{2+}]_i$ changes during single bursts should easily be resolved. Therefore, it seems more likely that the frequency discrepancy is due to the fact that $[Ca^{2+}]_i$ was analyzed in isolated cells, whereas membrane potential has been measured in electrically coupled β-cells within pancreatic islets (12, 18). It has been argued that ion channel sharing between tightly coupled multicellular aggregates is required to obtain the characteristic burst pattern, because single ion channel openings or closings would have too great effects on an isolated cell (34, 35). The data on glucose-induced $[Ca^{2+}]_i$ oscillations do not support the notion of a complete absence of bursts of membrane depolarization in isolated β-cells (35) but suggest that they are considerably slower than in electrically coupled β-cells.

The observation that tolbutamide could induce glucose-like oscillations of $[Ca^{2+}]_i$ strongly supports the idea that this phenomenon is tightly coupled to the bursts of membrane depolarization. Studies of electrically coupled β-cells within the pancreatic islet have revealed that tolbutamide, in the absence of glucose (13) or at subthreshold concentrations of the sugar (4), triggers bursts of membrane depolarization closely mimicking those induced by glucose. However, tolbutamide had no effect on the duration of each $[Ca^{2+}]_i$ peak during glucose-induced oscillations, although it has been found to prolong the bursts of membrane depolarization (4). This discrepancy may be understood if the membrane potential in electrically coupled cells is determined collectively, and tolbutamide excites passive cells within the functional syncytium. If ion channel sharing is important for the pattern observed when membrane potential is measured in electrically coupled cells, the variability of the $[Ca^{2+}]_i$ responses of isolated β-cells may be explained in terms of random variations in the numbers of sparse channels.

Although there are considerable similarities in the actions of glucose and tolbutamide, there are also differences. The sugar possesses a nutrient-specific $[Ca^{2+}]_i$-lowering effect, which starts earlier and at lower concentrations than the increasing component (9–12). Whereas the sulfonylurea response only involves a rise in $[Ca^{2+}]_i$, that triggered by a nutrient typically starts with lowering and is followed by an increase (9–12). In accordance with previous observations (36), the initial lowering action of glucose became even more pronounced in the presence of tolbutamide. The individual cell approach clearly indicated the power of this effect, by demonstrating a glucose-induced temporary return of $[Ca^{2+}]_i$ to the basal level. This $[Ca^{2+}]_i$-lowering action is probably due to stimulation of Ca^{2+} sequestration and outward transport. Apart from producing substrate for Ca^{2+}-ATPases, glucose can be expected to enhance Na^+/Ca^{2+} countertransport by lowering intracellular sodium (37, 38). Sulfonylureas, which increase the β-cell content of sodium (38, 39), should consequently counteract Ca^{2+} removal from the cytoplasm. Indeed, the increases in $[Ca^{2+}]_i$ were found to be more rapid during oscillations induced by tolbutamide than in those generated by glucose. However, there was no apparent difference between the decreases in $[Ca^{2+}]_i$.

In a recent study, it was shown that leucine but not arginine induces the characteristic large amplitude oscillations of $[Ca^{2+}]_i$ in a glucose-dependent manner (40). Also, in these cases, the oscillations occurred only under conditions known to be associated with bursts of membrane depolarization. The present data reinforce the idea that the oscillations of $[Ca^{2+}]_i$ and bursts of depolarization are different reflections of a single phenomenon. The ultimate verification or refutation of this concept will have to await technological advances allowing parallel measurements of $[Ca^{2+}]_i$ in electrically coupled β-cells and/or analysis of the electrophysiological characteristics of individual β-cells at physiological temperature.

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