Modalities of Gliclazide-induced Ca^{2+} Influx into the Pancreatic B-Cell

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SUMMARY

The hypoglycemic sulfonylurea gliclazide stimulated ^{45}Ca efflux and insulin release from prelabeled and perifused pancreatic rat islets, whether in the absence or presence of glucose (8.3 mM). The gliclazide-induced increase in ^{45}Ca efflux is thought to reflect a stimulation of ^{40}Ca influx into islet cells; it is suppressed in the absence of extracellular Ca^{2+} or in the presence of the organic Ca^{2+} -antagonist verapamil. In the absence of glucose, the ED_{50} for the inhibitory action of verapamil on gliclazide-stimulated ^{45}Ca efflux was almost identical to that found when the process of ^{40}Ca-^{45}Ca exchange was stimulated by an increase in the extracellular concentration of K^+, a procedure that leads to the depolarization of the plasma membrane. In the presence of glucose, however, the cationic response to gliclazide displayed an increased sensitivity toward the inhibitory action of verapamil. It is proposed that hypoglycemic sulfonylureas facilitate Ca^{2+} inflow into islet cells mainly through voltage-sensitive Ca^{2+} channels. In the presence of glucose, however, the stimulant action of hypoglycemic sulfonylureas upon Ca^{2+} entry into islet cells may entail a modality of Ca^{2+} transport not identical to that evoked by depolarization of the plasma membrane. **DIABETES 31:1010–1015, November 1982.**

Hypoglycemic sulfonylureas, like many other insulinotropic agents (including glucose), are thought to stimulate the release of insulin by provoking an accumulation of Ca^{2+} in the pancreatic B-cell.[1–3] Sulfonylureas may cause this intracellular accumulation by stimulating the inflow of Ca^{2+} through opened voltage-sensitive Ca^{2+} channels.[3,4] Indeed, tolbutamide and other sulfonylureas depolarize the B-cell,[5,6] an effect that has been attributed to the capacity of these drugs to reduce K^+ outflow from islet cells.[3,7] However, the gating of voltage-sensitive Ca^{2+} channels may not represent the sole mechanism by which sulfonylureas stimulate the entry of Ca^{2+} into the B-cell.[8] Thus, in the presence of an intermediate glucose concentration (8.3 mM), tolbutamide was reported to increase Ca^{2+} inflow into the B-cell while provoking at the same time a paradoxical increase in K^+ outflow from perifused islets.[8] As an alternative hypothesis, it has been proposed that sulfonylureas may increase Ca^{2+} inflow by acting synergistically with native ionophores possibly responsible for the transport of Ca^{2+} and/or other cations across the B-cell membrane.[9] This view is consistent with the knowledge that sulfonylureas display the ability to translocate cations across hydrophobic domains[10] and both artificial and living membranes.[11,12] In the artificial systems, sulfonylureas act synergistically with either the antibiotic ionophore A23187 or native ionophores extracted from the islets in mediating Ca^{2+} transport.[13,14]

In a recent work from this laboratory, the organic Ca^{2+} -antagonist verapamil was used as a tool to investigate the mechanisms by which glucose stimulates Ca^{2+} influx into the B-cell.[15] The glucose-induced increase in both ^{45}Ca efflux and insulin release from perifused islets displayed a much lesser sensitivity toward the inhibitory action of verapamil than that evoked by depolarization of the plasma membrane due to a rise in the extracellular concentration of K^+. This led us to suggest that B-cells, like smooth muscle cells,[16,17] are equipped with both voltage-sensitive and insensitive Ca^{2+} channels, and that glucose interacts with both types of channels to increase Ca^{2+} influx into the B-cells.[15]

In the present study, we have established the sensitivity toward verapamil of gliclazide-stimulated islets to gain further insight into the mode of action of hypoglycemic sulfonylureas on Ca^{2+} inward transport into islet cells.

MATERIAL AND METHODS

All experiments were performed with islets isolated by the collagenase technique[18] from the pancreas of fed albino rats. The media used for incubating, washing, or perifusing the islets consisted of a Krebs-Ringer bicarbonate-buffered

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solution supplemented with 0.5% (w/v) dialyzed albumin (Fraction V; Sigma Chemicals Co., St. Louis, Missouri) and equilibrated against a mixture of O_2 (95%) and CO_2 (5%). Some media contained no $CaCl_2$ and were enriched with 0.5 mM EGTA [ethylene glycol-bis-(β aminoethyl ether)-N,N'-tetraacetic acid]. The media also contained, as required, glucose, verapamil (Veride S.A., 1920 Diegem, Belgium), and gliclazide. Gliclazide was a gift from Servier Benelux. The method used for the measurement of ^{45}Ca efflux and insulin release from perifused islets has been described in detail elsewhere.[19]

All results are expressed as the mean (± SE) together with the number of individual experiments. The magnitude of the gliclazide-induced increase in ^{45}Ca efflux was estimated from the differences in ^{45}Ca efflux between the highest value seen after addition of gliclazide and the mean basal value found within the same experiment between 40 and 44 min of perifusion. The increase in insulin release was estimated in each individual experiment from the integrated output of insulin observed during stimulation, after correction for basal output (taken as the mean value for insulin release found within the same experiment between 39 and 44 min of perifusion).

RESULTS

Effect of gliclazide on ^{45}Ca efflux and insulin release from perifused islets. The sulfonylurea gliclazide (25 μg/ml) provoked a rapid and sustained increase in both ^{45}Ca efflux and insulin release from islets perifused either in the absence (Figures 1 and 2, left panels) or the presence of 8.3 mM glucose (Figure 3, left panels). Both the secretory and cationic responses to gliclazide were rapidly reversible on removal of the drug from the perifusate. The gliclazide-induced increase in ^{45}Ca efflux was completely suppressed in the absence of extracellular Ca^{2+} (Figure 1). When gliclazide was used at a lower concentration (1 μg/ml), the increases in both ^{45}Ca efflux and insulin release evoked by the sulfonylurea in the presence of 8.3 mM glucose were slightly, but not significantly, lower than those observed at the high concentration (25 μg/ml) of the drug (Table 1 and Figure 4).

Effect of glucose and K^+ on ^{45}Ca efflux and insulin release. For the purpose of comparison, control experiments were performed in which a rise in the concentration of glucose (from 0 to 8.3 mM) or K^+ (from 5 to 20 mM) was used to stimulate ^{45}Ca efflux and insulin release. The results of these experiments were illustrated in a prior publication (see Figures 4 and 5 in ref. 15). The absolute values for the increment in ^{45}Ca efflux and insulin release evoked by either glucose or K^+ are given in Table 1.

Effect of verapamil on gliclazide-induced increase in ^{45}Ca efflux and insulin release from perifused islets. Verapamil, when present since the onset of the perifusion, provoked a dose-related inhibition of the gliclazide-induced increases in insulin release and ^{45}Ca efflux from islets perifused either in the absence (Figure 2) or the presence (Figures 3 and 4) of 8.3 mM glucose.

The gliclazide-induced cationic and secretory responses displayed a high sensitivity toward the inhibitory effect of verapamil (Figure 5). In the absence of glucose, the sensitivity to increasing concentrations of verapamil was almost identical whether gliclazide or K^+ was used to stimulate

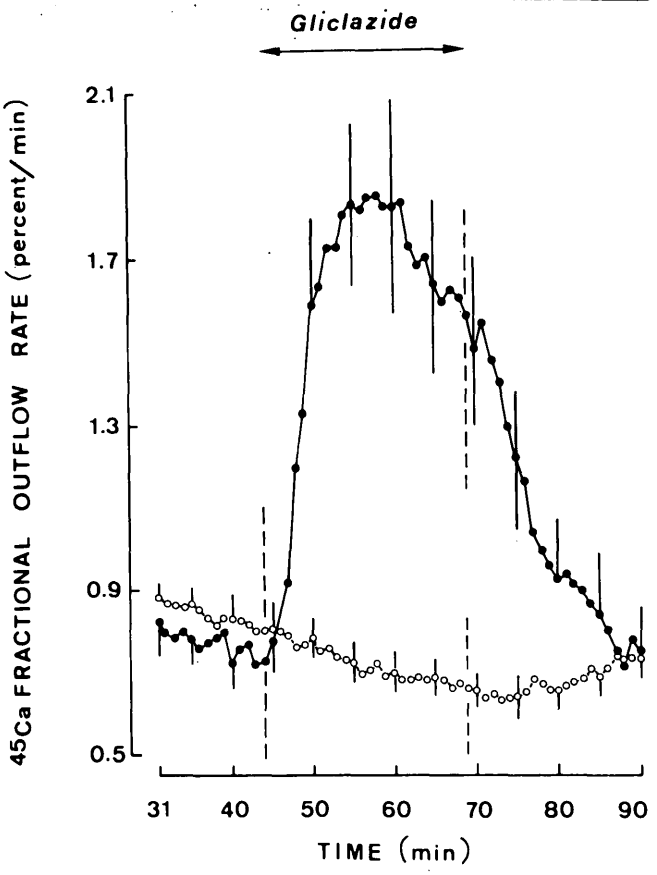


FIGURE 1. Effect of gliclazide (25 μg/ml) on ^{45}Ca efflux from islets perifused in the presence of 1 mM Ca^{2+} (●—●) or in a medium deprived of Ca^{2+} and enriched with 0.5 mM EGTA (○---○). Basal media contained no glucose. Mean values (± SE) for ^{45}Ca efflux are expressed as a fractional outflow rate[32] and refer to either 6 (presence of extracellular Ca^{2+}) or 12 (absence of extracellular Ca^{2+}) individual experiments.

^{45}Ca efflux (Figure 5).[15] The sensitivity to verapamil was significantly higher than that observed when a rise in the glucose concentration from 0 to 8.3 mM was used to stimulate ^{45}Ca efflux (Figure 5).[15] Covariance analysis indicated that the slopes of the regression lines characterizing the effect of verapamil on the cationic responses evoked by gliclazide or K^+ from glucose-deprived islets and by a rise in the glucose concentration from 0 to 8.3 mM were not significantly different from one another ($P > 0.1$). However, these regression lines differed markedly in their elevation. The differences in elevation were highly significant ($P < 0.005$ or less) except for the two regression lines characterizing the responses to gliclazide and K^+, respectively ($P > 0.25$). The latter two lines could thus be considered as virtually identical. In response to either gliclazide or K^+, the ED_{50} for the inhibitory action of verapamil was close to 5–7 μM. The ED_{50} for the inhibitory effect of verapamil upon the increase in ^{45}Ca efflux evoked by a rise in the glucose concentration from 0 to 8.3 mM amounted to 27 μM.

When the islets were perifused throughout in the presence of 8.3 mM glucose, the gliclazide-induced increase in ^{45}Ca efflux displayed a very high sensitivity toward the effect of verapamil (Figure 5). Indeed, the elevation of the regression line characterizing the effect of verapamil upon the cationic response evoked by gliclazide in the presence of 8.3 mM glucose was significantly lower ($P < 0.005$) than

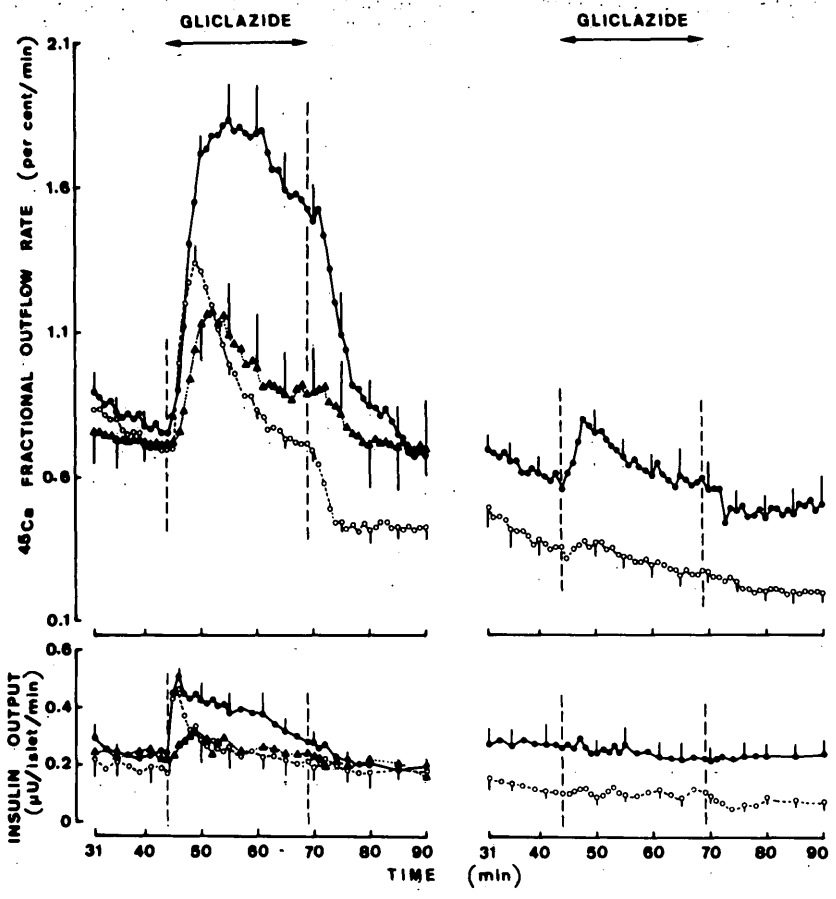


FIGURE 2. Effect of gliclazide (25 μg/ml) on ^{45}Ca efflux (upper panel) and insulin release (lower panel) from islets perifused in the absence (●—●, left panels) or presence of 5 μM (○---○, left panels), 10 μM (▲ · · ·▲, left panels), 20 μM (●—●, right panels), or 40 μM (○---○, right panels) verapamil. Basal media contained Ca^{2+} (1 mM) and no glucose. Mean values (± SE) for ^{45}Ca efflux are expressed as a fractional outflow rate and refer in each case to 4–7 individual experiments. Mean values (± SE) for insulin release are expressed in μU/islet/min and refer in each case to the same individual experiments.

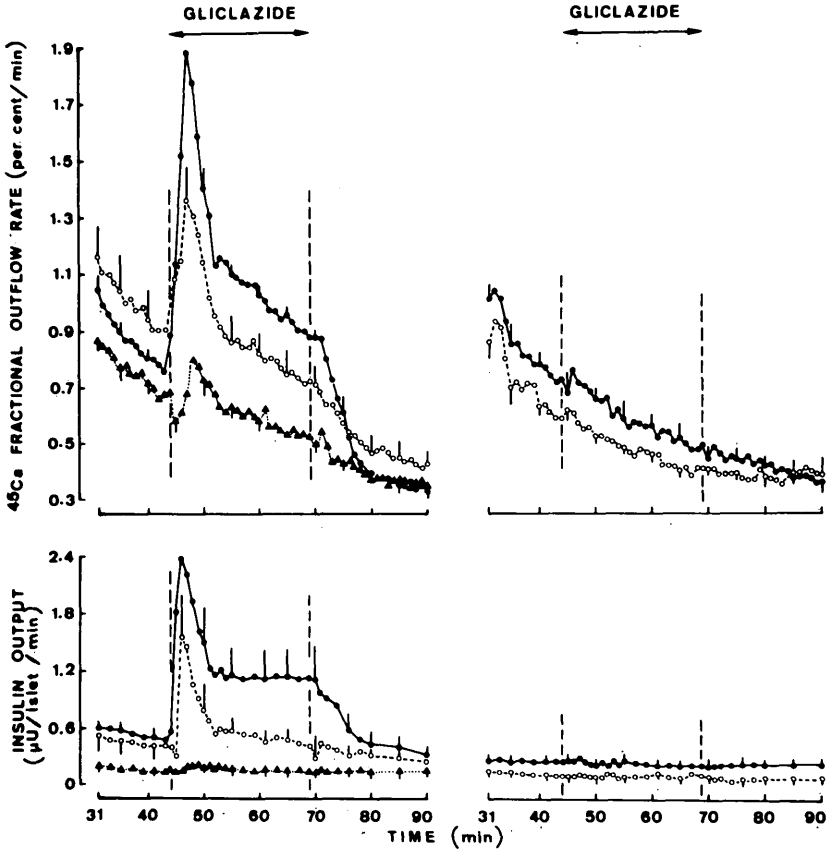


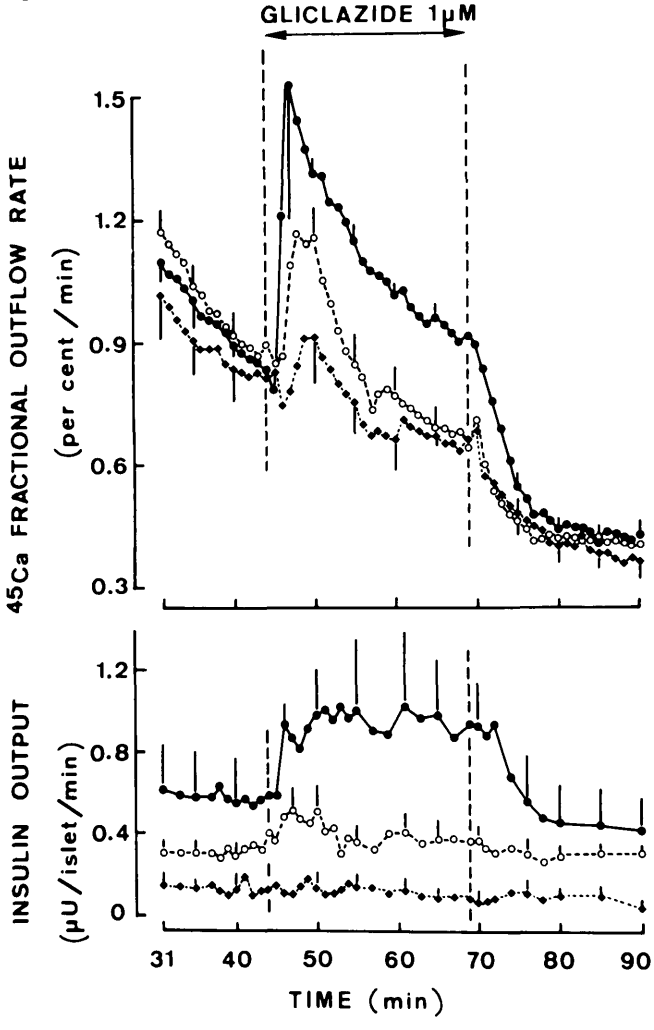
FIGURE 3. Effect of gliclazide (25 μg/ml) on ^{45}Ca efflux (upper panels) and insulin release (lower panels) from islets perifused in the absence (●—●, left panels) or presence of 5 μM (○---○, left panels), 10 μM (▲ · · ·▲, left panels), 20 μM (●—●, right panels), or 40 μM (○---○, right panels) verapamil. Basal media contained Ca^{2+} (1 mM) and glucose (8.3 mM). Mean values (± SE) for ^{45}Ca efflux and insulin release are expressed as in Figure 2 and refer in each case to four individual experiments.

TABLE 1
Mean control values (± SEM) for the increments in ^{45}Ca efflux and insulin output evoked by distinct secretagogues in islets perifused in the absence of verapamil

Glucose concentration (min 0–90)	Stimulus (min 45–69)	Increment in ^{45}Ca efflux (%/min)	Increment in insulin output (μU/min/islet)
Nil	Gliclazide (25 μg/ml)	1.25 ± 0.13 (10)	0.22 ± 0.06 (6)
8.3 mM	Gliclazide (25 μg/ml)	0.97 ± 0.12 (8)	0.65 ± 0.20 (7)
8.3 mM	Gliclazide (1 μg/ml)	0.87 ± 0.20 (4)	0.48 ± 0.08 (4)
Nil	K^+ (20 mM)	1.30 ± 0.07 (8)	0.17 ± 0.04 (8)
Nil	Glucose (8.3 mM)	0.58 ± 0.07 (8)	0.20 ± 0.03 (8)

that observed in the absence of the sugar. In the presence of 8.3 mM glucose, the ED_{50} for the inhibitory action of verapamil on the gliclazide-induced increase in ^{45}Ca efflux was close to 2 μM. In the presence of glucose (8.3 mM), the percent inhibitory effect of verapamil (5 and 10 μM) on the cationic and secretory responses to gliclazide was essentially the same, whether the sulfonylurea was used in a low (1 μg/ml) or high (25 μg/ml) concentration. Indeed, neither the slopes nor the elevations of the regression lines characterizing the inhibitory effect of verapamil were significantly different at these two concentrations of gliclazide (data not shown).

FIGURE 4. Effect of gliclazide (1 μg/ml) on ^{45}Ca efflux (upper panel) and insulin release (lower panel) from islets perifused in the absence (●—●) or presence of 5 μM (○---○) or 10 μM (◆ · · · ◆) verapamil. Basal media contained Ca^{2+} (1 mM) and glucose (8.3 mM). Mean values (± SE) for ^{45}Ca efflux and insulin release are expressed as in Figure 2 and refer in each case to four individual experiments.



Comparison between the effects of verapamil on the cationic and secretory responses to distinct secretagogues. As illustrated in Figure 5, the sensitivity toward increasing concentrations of verapamil of islets stimulated by glucose, K^+, and gliclazide (in the presence of glucose), respectively, displayed the same hierarchy, whether such a sensitivity was judged from the increase in ^{45}Ca efflux or the increment in insulin output. In both cases, the islets stimulated by glucose (8.3 mM) were the least sensitive to verapamil, whereas the islets stimulated by gliclazide in the presence of glucose were the most sensitive to the inhibitory action of the Ca^{2+}-antagonist. It should be noted, however, that the concentration of verapamil required to cause a given percentage inhibition of insulin release was always lower than that required to cause the same percentage inhibition of ^{45}Ca efflux, whatever secretagogue was used to stimulate the B-cell. The differences between such concentrations were of the same order of magnitude for distinct secretagogues and, as an average, corresponded to a twofold change (2.1 ± 0.2) in verapamil concentration. The existence of such a difference is in good agreement with the knowledge that the entry of Ca^{2+} in islet cells must be increased beyond a critical threshold value to stimulate insulin release.[19]

DISCUSSION

Up to five different modalities are currently considered to account for Ca^{2+} inflow across cell membranes.[20] Although the molecular identity of each transporter remains to be elucidated, the identification of distinct pathways is based, inter alia, on their respective responsiveness to suitable agonists or antagonists. For instance, in different tissues, e.g., in smooth muscle cells, organic calcium-antagonists such as verapamil and D-600 were used to distinguish between voltage-sensitive and insensitive Ca^{2+} channels, the latter being more resistant than the former to such antagonists.[16,17] Although this pharmacologic approach may not be sufficient to elucidate the precise modality of Ca^{2+} inward transport, we have recently proposed that the two types of Ca^{2+} channels may also be present in islet cells.[15] In this prior study, as in the present one, the response to distinct insulin secretagogues was tested at increasing concentrations of verapamil. Although, in pancreatic islets, verapamil may exert side effects on lactic acid output[21] and K^+ conductance,[22] its inhibitory action on insulin release appears indeed attributable to altered Ca^{2+} inflow into the islet cells.[22] It should be realized, however, that verapamil does not act solely on voltage-sensitive or insensitive Ca^{2+} channels. For instance, in pancreatic islets, verapamil also suppresses the secretory response evoked by the antibiotic ionophore A23187,[23] a situation reminiscent of the inhibitory action of calcium-antagonists upon ionophore-mediated Ca^{2+} translocation in artificial systems.[24] The relevance of the latter findings to the present study is evident, since it was recently hypothesized that the ionophoretic capacity of

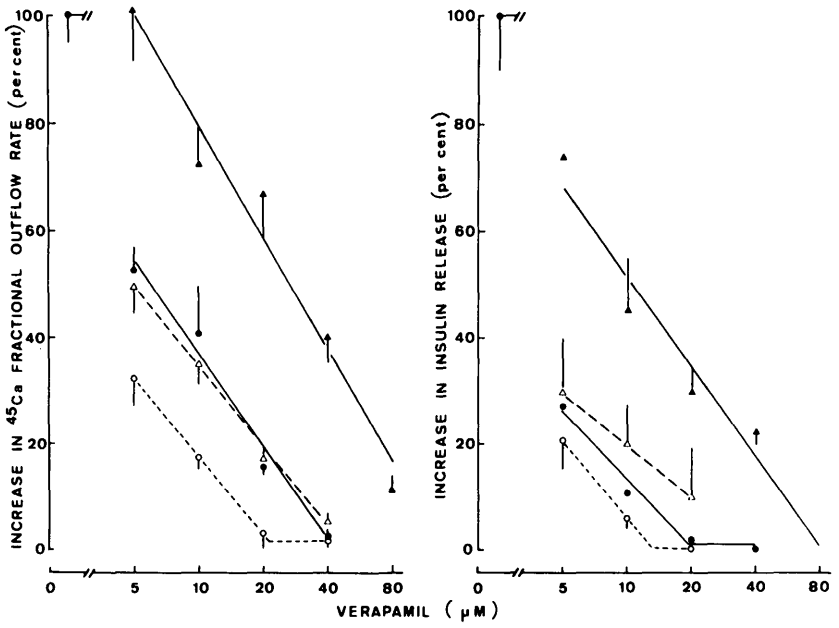


FIGURE 5. Dose-action relationships for the effect of verapamil on the increases in ^{45}Ca efflux (left panel) and insulin release (right panel) induced by gliclazide from islets perifused in the absence (●—●) or presence of 8.3 mM glucose (○---○), by a rise in the glucose concentration from 0 to 8.3 mM (▲—▲), and by a rise in the K^+ concentration from 5 to 20 mM (△---△). Mean values (±SE) are expressed as a percentage of the mean control value obtained within each series of experiments in the absence of verapamil (see Figures 2 and 3 and ref. 15). Absolute values for the control data collected in the absence of verapamil are listed in Table 1.

hypoglycemic sulfonylureas may account, in part at least, for their insulinotropic property.[9,10] With this background information in mind, the present study was undertaken to gain further insight on the pathways involved in the stimulant action of hypoglycemic sulfonylureas on Ca^{2+} inflow in the islet cells.

The effect of gliclazide on ^{45}Ca efflux. The present data confirm that gliclazide provokes a dramatic increase in ^{45}Ca efflux from preloaded and perifused islets.[25] This increase in ^{45}Ca efflux, like that induced by glucose, is suppressed in the absence of extracellular Ca^{2+} and inhibited in the presence of verapamil. Therefore, it is likely to reflect stimulated Ca^{2+} entry in islet cells and to correspond to a process of Ca-Ca exchange in which influent ^{40}Ca displaces ^{45}Ca from intracellular binding sites, as previously documented in the case of stimulation by glucose and K^+.[26–29] The increase in ^{45}Ca efflux induced by gliclazide was therefore taken, in the present study, as an index of stimulated ^{40}Ca entry into the B-cell.

In a previous work, gliclazide was found to exert a biphasic effect upon ^{45}Ca efflux from islets perifused in the absence of extracellular Ca^{2+}. It consisted of a rapid but transient inhibition followed by a late and modest increase in ^{45}Ca efflux.[25] Such a biphasic effect of gliclazide on ^{45}Ca efflux from Ca^{2+}-deprived islets was not confirmed in the present and much larger series of experiments. Hellman also failed to observe an effect of sulfonylureas on ^{45}Ca efflux from islets perifused in the absence of extracellular Ca^{2+}.[4]

Effect of verapamil on gliclazide-stimulated ^{45}Ca efflux in the absence of glucose. Islets stimulated by gliclazide in the absence of glucose displayed a high sensitivity toward the inhibitory effect of verapamil. Such a sensitivity was strictly superimposable to that observed in islets stimulated by a rise in the extracellular K^+ concentration, and much greater than that observed in islets stimulated by a rise in the glucose concentration from 0 to 8.3 mM. The ED_{50} for the inhibitory effect of verapamil on ^{45}Ca efflux was almost identical (5–7 μM) in the K^+- and gliclazide-stimulated islets, and four times lower than in the islets exposed to 8.3 mM glucose (27 μM). It is well established that a rise in the concentration of extracellular K^+ provokes depolarization of the plasma membrane and, hence, causes the gating of voltage-sensitive Ca^{2+} channels in the islet cells.[28–30] The finding that K^+- and gliclazide-stimulated islets, respectively, display a comparable sensitivity toward verapamil suggests that gliclazide also increases Ca^{2+} influx into the B-cell by gating voltage-sensitive Ca^{2+} channels.

The latter view is compatible with the knowledge that hypoglycemic sulfonylureas indeed provoke B-cell depolarization,[5,6] an effect apparently attributable, in part at least, to a decrease in K^+ conductance.[3,7] The present findings are also in good agreement with previous studies on ^{45}Ca net uptake by islets, indicating that islets stimulated by glucose are much more resistant to the inhibitory effect of organic Ca^{2+}-antagonists than islets stimulated by hypoglycemic sulfonylureas.[21,31]

Incidentally, it is unlikely that the difference in the response toward verapamil of K^+-, gliclazide-, and glucose-stimulated islets reflects a difference in the intracellular handling of ^{45}Ca under these three experimental conditions. Such a difference, if it exists, may affect the quantitative relationship between influent ^{40}Ca and effluent ^{45}Ca in response to distinct secretagogues. However, in response to a given secretagogue, the dose-related inhibitory effect of verapamil on stimulated ^{45}Ca efflux reflects the decrease in ^{40}Ca inflow. Hence, the sensitivity to verapamil can be adequately assessed by expressing the results obtained in the presence of the Ca^{2+}-antagonist *relative* to the control value found with the same secretagogue in the absence of verapamil. Likewise, the difference in sensitivity toward verapamil cannot be ascribed to differences in the magnitude of the cationic response to each secretagogue as seen in the absence of verapamil, since there is no obvious link between the sensitivity to verapamil and the magnitude of the control cationic response.

Effect of verapamil on gliclazide-stimulated ^{45}Ca efflux in the presence of glucose. Gliclazide also increased ^{45}Ca efflux and insulin release from islets perifused in the presence of 8.3 mM glucose (Figures 3 and 4). Under the latter

condition, the increase in ^{45}Ca efflux also displayed a high sensitivity to verapamil, whatever the concentration of gliclazide (1 or 25 μg/ml) used to stimulate insulin release. This could indicate that, under a condition that mimicks the situation found in vivo when sulfonylureas are used for a therapeutic purpose, gliclazide again stimulates Ca^{2+} influx into islet cells mainly through voltage-sensitive Ca^{2+} channels. The sensitivity toward verapamil of the ionic response to gliclazide, however, was significantly higher in the islets perifused in the presence of glucose than in glucose-deprived islets; the ED_{50} for the inhibitory effect of verapamil on gliclazide-stimulated ^{45}Ca efflux averaged 2 μM in islets perifused in the presence of 8.3 mM glucose. Such a high sensitivity could be taken as an indication that, in the presence of glucose, when the B-cell membrane is already depolarized during each burst of spike, gliclazide stimulates Ca^{2+} influx in islet cells by a third pathway or modality, characterized by a higher sensitivity to verapamil than the voltage-dependent Ca^{2+} channels. For instance, this third modality could reflect the capacity of hypoglycemic sulfonylureas to act as ionophores in cooperativity with native ionophores.[9,14] Although highly speculative, the latter view is consistent with the knowledge that organic Ca^{2+}-antagonists are potent inhibitors of the process of sulfonylurea-mediated Ca^{2+} translocation in artificial systems.[10]

In conclusion, the present study suggests that, in the absence of glucose, the sulfonylurea gliclazide interacts mainly with voltage-sensitive Ca^{2+} channels to increase Ca^{2+} influx in islet cells. In the presence of glucose, however, the stimulant action of gliclazide upon Ca^{2+} inflow into islet cells may involve a different modality of Ca^{2+} transport, characterized by a higher sensitivity toward the inhibitory action of verapamil.

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