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Tolbutamide Stimulation of ^{45}Ca Fluxes in Microdissected Pancreatic Islets Rich in β-Cells

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SUMMARY

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Tolbutamide and glucose were compared with regard to their actions on ^{45}Ca fluxes in pancreatic islets microdissected from *ob/ob* mice. In contrast to an analogue lacking effects on insulin release, tolbutamide stimulated the intracellular net uptake of ^{45}Ca as well as the efflux of the isotope. The magnitude of both effects equaled those produced by 20 mM glucose. The tolbutamide stimulation of ^{45}Ca efflux was more rapidly established than that with glucose; combination of glucose and tolbutamide did not increase the efflux more than with either alone. Comparisons of the ^{45}Ca efflux from islets loaded in the presence of tolbutamide or glucose suggested that these compounds had different effects of the intracellular distribution of the incorporated ^{45}Ca. The action of tolbutamide diverged from that of glucose also in not inhibiting the ^{45}Ca efflux during perifusion with Ca^{2+}-deficient medium. It is likely that the tolbutamide effect on the β-cell handling of ^{45}Ca is essentially due to opening of potential-dependent channels with resulting increase in the entry of Ca^{2+}.

INTRODUCTION

Accumulating evidence suggests that the recognition of tolbutamide as an insulin secretagogue follows from its interaction with the plasma membrane of the pancreatic β-cells (1–3). Evidently Ca^{2+} has a key function in the subsequent events leading to the discharge of insulin by exocytosis of the secretory granules. The requirement of extracellular Ca^{2+} for the tolbutamide stimulation of insulin release is well established (4). Furthermore, tolbutamide has been found to stimulate ^{45}Ca uptake into isolated pancreatic islets from both mice (5) and rats (6).

In the present study, tolbutamide and glucose were compared with regard to their actions on ^{45}Ca fluxes in β-cell-rich pancreatic islets microdissected from *ob/ob* mice. Although both compounds proved to be equally effective promotors of the entry of Ca^{2+}, differences seem to exist with regard to the intracellular distribution of the incorporated calcium.

MATERIALS AND METHODS

Adult *ob/ob* mice were taken from a non-inbred colony (7) and were fasted overnight. The animals were killed by decapitation and the islets were isolated by microdissection. The basal medium used for the isolation of the islets and the subsquent studies of their ^{45}Ca handling was a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer, pH 7.4, containing cations in physiological (extracellular) concentrations with Cl^- as the sole anion (8).

Previously described procedures were used for measuring the uptake and efflux of ^{45}Ca at 37° (9–11). In the uptake studies, subsequent washing with a cold solution of La^{3+} made possible estimates of the amounts of ^{45}Ca incorporated intracellularly during 20-min periods. When the dynamics of ^{45}Ca efflux and insulin release were studied, the islets were loaded for 90 min with 1.28 mM ^{45}Ca and subsequently perifused in parallel from separate reservoirs with a medium containing 1 mg/ml of albumin. The islet content of ^{45}Ca at a given moment during efflux was obtained by adding the radioactivity subsequently released to that remaining in the islets at the end of the experiment. Insulin was measured radioimmunologically by using crystalline mouse insulin as a reference. As a final step in the uptake and efflux studies, the islets were freeze-dried overnight and weighed on a quartz fiber balance.

Experimental data were expressed per kilogram of islet dry weight, assuming the same specific radioactivity for ^{45}Ca as in the loading medium. Since isotopic equilibrium was not attained in the islets under study, the data cannot be used for estimating absolute changes in different calcium pools. Statistical significance of effects was assessed from the differences between paired test and control data using the two-tailed Student's distribution.

Throughout the experiments, reagents of analytical

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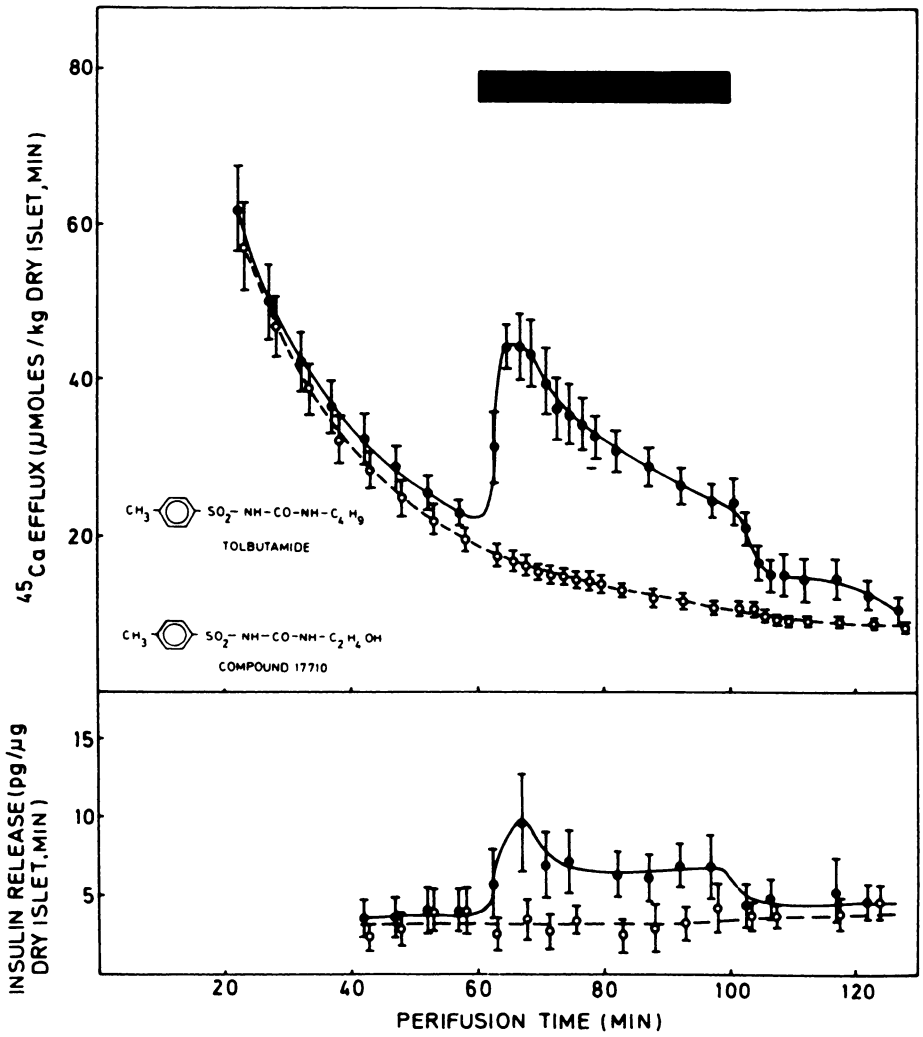

FIG. 1. *Effects of tolbutamide and compound 17710 on ^{45}Ca efflux and insulin release*

The islets were labeled with ^{45}Ca in the presence of 20 mM glucose and perifused with glucose-free medium containing 2.56 mM Ca^{2+}. During the period indicated by the *horizontal black bar*, the islets were exposed to either 100 μM tolbutamide (●) or 100 μM compound 17710 (○). Mean values ± standard error of the mean for four experiments.

grade and deionized water were used. NEN Chemicals GmbH (Dreieich, Federal Republic of Germany) supplied ^{45}Ca. [^{125}I]Insulin, as well as tolbutamide with its inactive analogue 17710 (structural formulae are given in Fig. 1), were products of Hoechst A. G. (Frankfurt/Main, Federal Republic of Germany).

RESULTS

Tolbutamide differed from the analogue 17710 in that it significantly influenced the calcium fluxes in the pancreatic islets. Whereas 100 μM tolbutamide markedly stimulated both the intracellular net uptake of ^{45}Ca (Table 1) and the efflux during perfusion with a glucose-free medium containing 2.56 mM Ca^{2+} (Fig. 1, *upper panel*), these effects could not be reproduced with the compound 17710. During the tolbutamide stimulation of ^{45}Ca efflux, there was a moderate increase of insulin release with an initial peak coinciding with that for the washout of radioactivity (Fig. 1, *lower panel*).

The effect on ^{45}Ca efflux of different concentrations of tolbutamide are shown in the *left panel* of Fig. 2. There was a marked stimulation of ^{45}Ca efflux already in the presence of 30 μM tolbutamide. Rise of the tolbutamide concentration to 1 mM produced only a small increase in addition to that seen with 100 μM. The sulfonylurea stimulation of ^{45}Ca efflux required the presence of a certain concentration of extracellular Ca^{2+}. However, contrary to the action of glucose, the addition of tolbutamide did not result in an inhibition of ^{45}Ca efflux in a Ca^{2+}-deficient medium (Fig. 2, *right panel*). The pattern of glucose inhibition remained unaffected when 20 mM glucose was combined with 100 μM tolbutamide.

Tolbutamide was as effective as 20 mM glucose in promoting the intracellular uptake of ^{45}Ca (Table 1) or in increasing the efflux of radioactivity from islets loaded with this isotope (Fig. 3, *left panel*), the stimulatory effect of tolbutamide on ^{45}Ca efflux being more rapidly established than that of glucose. The efflux rate increased by 105 ± 16% during the first 2-min period after exposure of the islets to 100 μM tolbutamide when the stimulatory effect of 20 mM glucose was still lacking. The simultaneous exposure to 100 μM tolbutamide and 20 mM glucose

TABLE 1

Intracellular incorporation of ^{45}Ca into islets exposed to tolbutamide or compound 17710

After preliminary incubation in basal medium supplemented with 3 mM glucose, the islets were loaded for 20 min with 1.28 mM ^{45}Ca in media supplemented or not with test substances in the concentrations indicated. The radioactivity retained after washing with La^{3+} is expressed in terms of millimoles of calcium with the same specific activity as in the incubation medium. Mean values ± standard error of the mean for six separate experiments.

Compound added	Uptake of La^{3+}-nondisplaceable ^{45}Ca	
	Uptake observed	Effect of test substance
	mmoles/kg islet dry wt	
None (control)	2.98 ± 0.29	—
Compound 17710 (100 μM)	3.43 ± 0.20	+0.45 ± 0.34
Tolbutamide (100 μM)	4.35 ± 0.09	+1.37 ± 0.33[a]
Glucose (20 mM)	4.55 ± 0.29	+1.57 ± 0.30[a]

[a] $p < 0.01$

did not result in increased mobilization of ^{45}Ca beyond that obtained with glucose alone (Fig. 3, *right panel*). However, in the presence of glucose, there was a tendency to a delay in the maximal response to tolbutamide.

The tolbutamide action on ^{45}Ca efflux differed from that on insulin release in that it was dependent on the composition of the medium employed during the loading with ^{45}Ca. Removal of glucose from the loading medium nearly abolished the tolbutamide stimulation of ^{45}Ca efflux (Fig. 4, *left panel*). Glucose stimulated ^{45}Ca efflux also from islets loaded in the presence of 1 mM tolbutamide. This effect was, however, considerably smaller than that recorded when the loading medium was supplemented with 20 mM glucose (Fig. 4, *right panel*).

Table 2 summarizes the results of another series of experiments comparing the radioactive efflux from islets loaded with ^{45}Ca in the presence or absence of tolbutamide or glucose. Whereas the ^{45}Ca incorporated in response to glucose was particularly well mobilized by this compound, there was no evidence that tolbutamide was more effective than glucose in promoting the efflux of the ^{45}Ca taken up when tolbutamide had been present in the loading medium.

DISCUSSION

The present data lend support to the view that sulfonylureas markedly influence the β-cell handling of Ca^{2+} (5, 6, 12, 13). A clear effect was noted with as little as 30

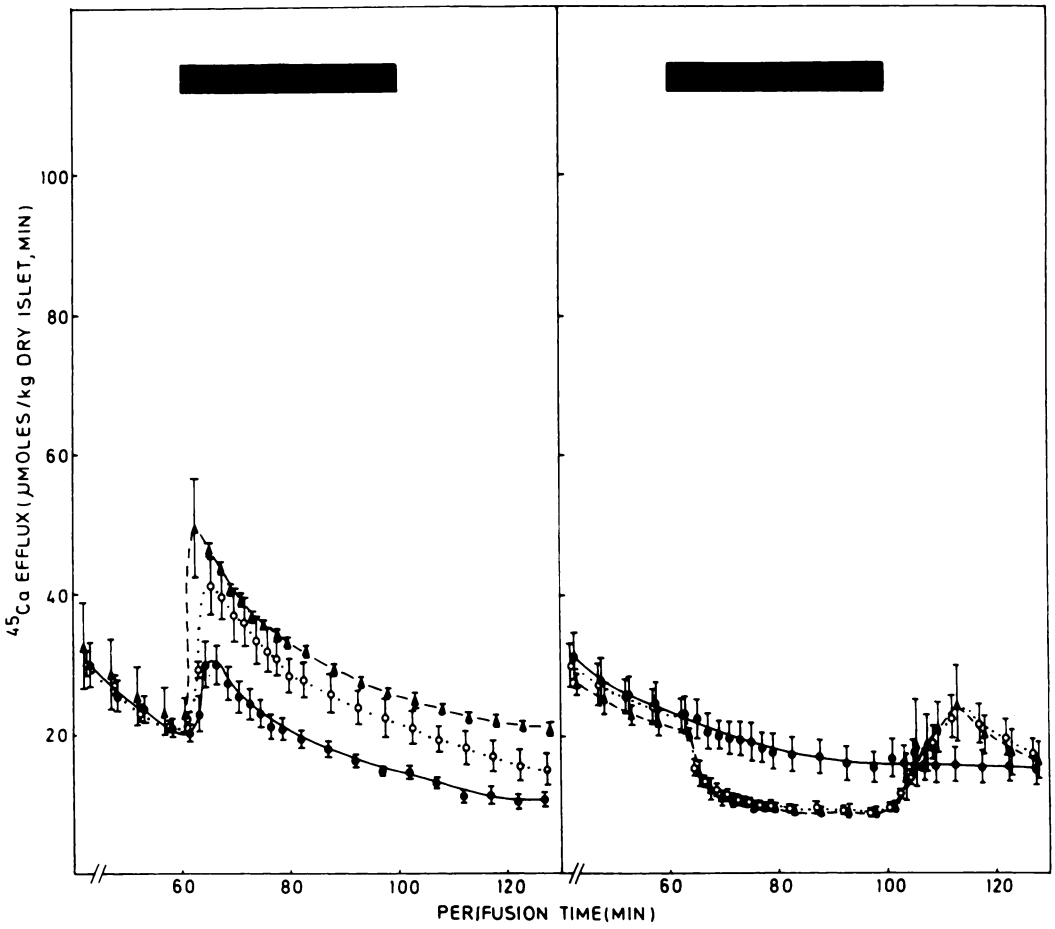

FIG. 2. *Effects of tolbutamide and glucose on ^{45}Ca efflux*

The islets were labeled with ^{45}Ca in the presence of 20 mM glucose. In the *left panel*, 30 μM (●), 100 μM (○), or 1 mM (▲) tolbutamide were added to a glucose-free perifusion medium containing 2.56 mM Ca^{2+} during the period indicated by the *horizontal black bar*. In the *right panel*, either 100 μM tolbutamide (●), 20 mM glucose (○), or a combination of these substances (▲) were added to a Ca^{2+}-deficient medium containing 0.5 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid as indicated by the *bar*. Mean values ± standard error of the mean for four experiments.

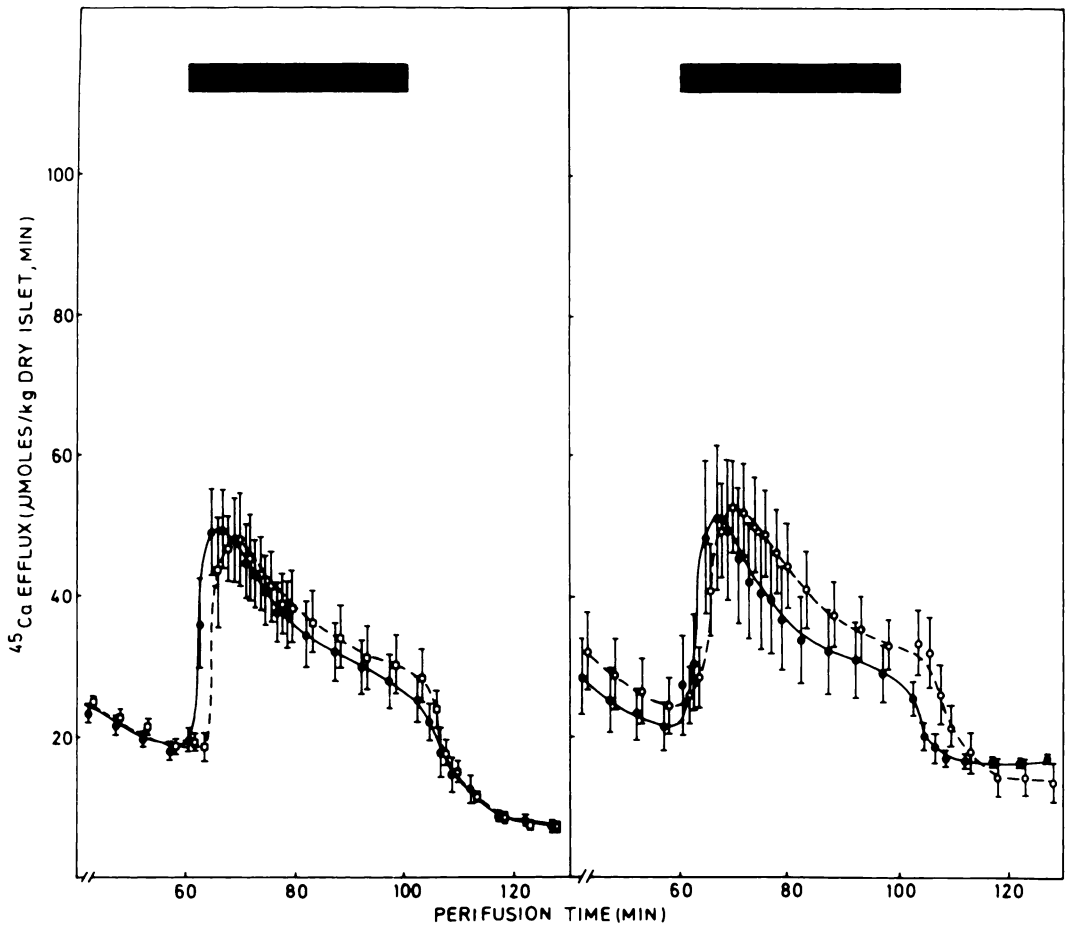

FIG. 3. *Effects of tolbutamide and glucose on ^{45}Ca efflux*

The islets were labeled with ^{45}Ca in the presence of 20 mM glucose and perifused with glucose-free medium containing 2.56 mM Ca^{2+}. In the *left panel*, either 1 mM tolbutamide (●) or 20 mM glucose (□) were introduced during the period indicated by the *horizontal black bar*. In the *right panel*, the corresponding additives were either 100 μM tolbutamide alone (●) or in combination with 20 mM glucose (○). Mean values ± standard error of the mean for four to five experiments.

μM tolbutamide (8.1 μg/ml), a concentration well below the serum levels reached in the clinical praxis (14). The tolbutamide effect on ^{45}Ca efflux was closely related to that on insulin release. Further evidence for the significance of the Ca^{2+} movements for the insulin-releasing action of tolbutamide was obtained from the experiments with its analogue 17710. The latter compound neither stimulated the transmembrane ^{45}Ca fluxes nor the release of insulin when added to a perifusion medium containing physiological concentrations of Ca^{2+}.

The promotion of ^{45}Ca efflux is by no means a unique effect of the therapeutically active sulfonylureas. It is well established that a similar effect can be obtained with glucose and other insulin secretagogues with depolarizing effects on the β-cells (11, 15–17). The glucose-induced stimulation of ^{45}Ca efflux has been attributed essentially to an exchange with the nonradioactive Ca^{2+} entering the β-cells after opening of potential-dependent channels (18, 19). It can be supposed that a similar mechanism operates in the case of tolbutamide. Like glucose, this sulfonylurea compound has been found to depolarize the β-cells (20), a phenomenon which might be related to a reduced K^+ permeability of the β-cells (6, 21). The similarities between the actions of tolbutamide and glucose became obvious when comparing the pattern of stimulated ^{45}Ca efflux. The maximal activity reached by tolbutamide was almost identical to that obtained after the addition of 20 mM glucose, a concentration which evokes optimal stimulation of ^{45}Ca washout (22). The glucose stimulation of ^{45}Ca efflux was somewhat delayed when compared to the effect produced by tolbutamide. Corresponding differences are known to exist for the insulin-releasing actions of these compounds (23, 24).

After the original observation that tolbutamide is distributed through the entire extracellular space in the islets of the *ob/ob* mice (1), the sulfonylurea stimulation of insulin release has been assumed to involve a direct interaction with the plasma membrane of the pancreatic β-cells. Later reports of an insulin-releasing effect of soluble dextran-conjugated tolbutamide (25) are difficult to evaluate in view of the observed instability of such conjugates (26). The target groups for sulfonylureas may consequently be embedded within the plasma membrane rather than located at the outer surface. It is pertinent to note that tolbutamide and other hypoglycemic sulfonylureas can translocate ^{45}Ca into an organic immiscible phase; a behavior reminiscent of that of genuine Ca^{2+} ionophores (27, 28). The significance of these findings will remain obscure until the experiments have been repeated in a system with two stationary water compartments (29, 30). The present data provide no support for the view that the observed alterations in the β-cell han-

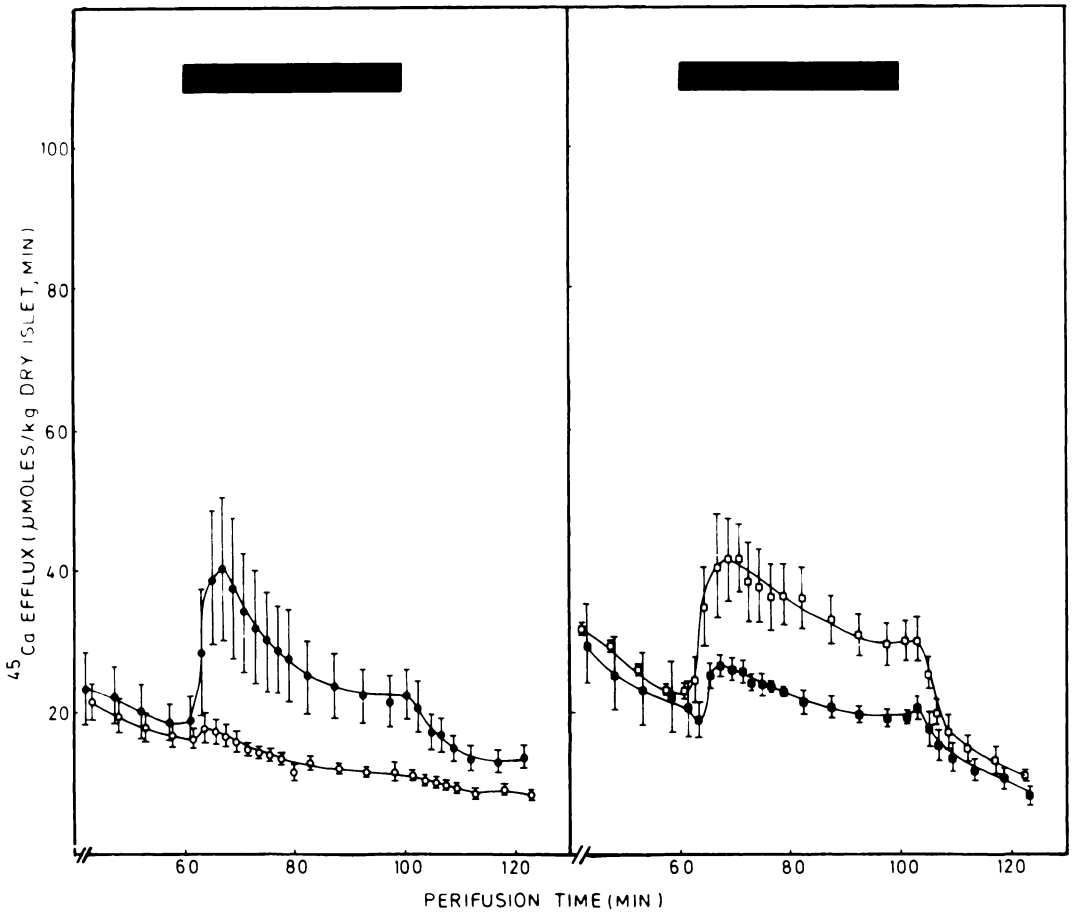

FIG. 4. *Effects of tolbutamide and glucose on ^{45}Ca efflux from islets labeled with the radioactive isotope in the presence of tolbutamide or glucose*

The experiments were performed with perifusion medium containing 2.56 mM Ca^{2+}. In the *left panel*, the islets were labeled in the absence (○) or presence (●) of 20 mM glucose and exposed to 100 μM tolbutamide during the period indicated by the *horizontal black bar*. In the *right panel*, the islets were labeled either in the presence of 1 mM tolbutamide (■) or 20 mM glucose (□) and exposed to 20 mM glucose during the period indicated by the *bar*. Mean values ± standard error of the mean for four experiments.

dling of Ca^{2+} are a consequence of an ionophoretic activity of tolbutamide. The increase of the tolbutamide concentration from 100 μM to 1 mM resulted only in minor stimulation of ^{45}Ca efflux during perifusion with physiological concentrations of Ca^{2+}. It was also evident that the simultaneous exposure to tolbutamide and glucose did not result in increased mobilization of ^{45}Ca beyond that obtained with glucose alone. Furthermore, if tolbutamide acted as a genuine Ca^{2+} ionophore in the isolated islets, it should be expected to promote the efflux of ^{45}Ca also in a Ca^{2+}-deficient perifusion medium (31).

In accordance with previous studies (6), tolbutamide was found to exhibit a rather weak insulin-releasing action when added to a glucose-free medium. Nevertheless, tolbutamide was as efficient as glucose in promoting the intracellular uptake of ^{45}Ca or in increasing the efflux of radioactivity from islets loaded with this isotope. It remains to be established why tolbutamide lacks the full capacity of glucose to initiate insulin release despite its being an equally potent stimulator of the Ca^{2+} entry into the β-cells. When considering this matter, attention should be paid to differences in their actions on the intracellular distribution of calcium as suggested from the comparison of the ^{45}Ca efflux from islets loaded either in the presence of tolbutamide or glucose.

TABLE 2

Stimulation of ^{45}Ca efflux from islets labeled with the radioactive isotope in the presence and absence of tolbutamide or glucose

The islets were labeled with ^{45}Ca in the presence or absence of 100 μM tolbutamide or 20 mM glucose and perifused with glucose-free media containing 2.56 mM Ca^{2+}. After 60 min of perifusion, either 100 μM tolbutamide or 20 mM glucose was added to the medium; the resulting peak of efflux is expressed in per cent of the value during the preceding 5-min period. Mean values ± standard error of the mean for four to five separate experiments.

Compound added to the loading medium	Compound added to the perifusion medium	Prestimulatory concentration of ^{45}Ca	Peak of ^{45}Ca efflux[a]
		mmoles/kg dry wt	
None	Tolbutamide (100 μM)	1.78 ± 0.16	13.0 ± 1.8
Tolbutamide (100 μM)	Tolbutamide (100 μM)	3.32 ± 0.28	52.6 ± 14.4
Glucose (20 mM)	Tolbutamide (100 μM)	4.21 ± 0.46	80.4 ± 13.2
None	Glucose (20 mM)	1.80 ± 0.40	8.7 ± 4.7
Tolbutamide (100 μM)	Glucose (20 mM)	4.43 ± 0.72	34.5 ± 7.9
Glucose (20 mM)	Glucose (20 mM)	4.37 ± 0.62	82.1 ± 14.8

[a] Percentage increase above prestimulatory value.

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