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## ORIGINALS

# The Stimulus-Secretion Coupling of Glucose-Induced Insulin Release XII. Effects of Diazoxide and Gliclazide upon $^{45}$Calcium Efflux from Perifused Islets

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*Summary.* The effect of diazoxide and gliclazide upon $^{45}$calcium efflux from perifused islets was investigated. In the absence of extracellular calcium and presence of glucose, diazoxide caused an immediate and sustained increase in $^{45}$calcium efflux. This facilitation of the outward transport of calcium might explain why diazoxide inhibits the stimulant action of glucose upon calcium accumulation in the beta cell. Under conditions known to abolish insulin release and the concomitant release of $^{45}$calcium, namely in the absence of extracellular calcium or in the presence of deuterium oxide, gliclazide caused an immediate but transient decrease in $^{45}$calcium efflux, this inhibition of the outward transport of calcium being observed both in the absence and presence of glucose and being followed by a significant rebound in $^{45}$calcium efflux. It is suggested that this biphasic change in $^{45}$calcium efflux could be due to a dual effect of gliclazide upon calcium handling by the beta cell, similar to that evoked by glucose and theophylline respectively.

*Key words*: insulin secretion, calcium, isolated islets, diazoxide, sulfonylurea, gliclazide, deuterium oxide.

The concentration of calcium in the cytosol of the beta cell is thought to regulate the rate of insulin release by controlling the activity of a microtubular-microfilamentous system involved in the migration and extrusion of secretory granules [1]. Thus, glucose apparently causes an accumulation of calcium in the beta cell [2] by inhibiting the outward transport of calcium across the cell membrane [3]. Theophylline might provoke an intracellular translocation of calcium from the vacuolar system into the cytosol [4]. Epinephrine was recently found to antagonize the effect of glucose and that of theophylline on insular calcium metabolism [5].

The aim of the present study is to investigate whether the inhibition of glucose-induced insulin secretion by diazoxide and the insulinotropic action of sulfonylurea might also be due to rapid changes in calcium handling by the beta cell. For this purpose, we have examined the influence of diazoxide and gliclazide, a new sulfonylurea [6], upon the efflux of $^{45}$calcium from perifused islets of Langerhans. Such an efflux is thought to correspond (i) to a release of $^{45}$calcium concomitant with insulin release and occuring at the time and site of emiocytosis; and (ii) to the transport of $^{45}$calcium across the intact membrane of the beta cell [3]. In order to study the latter movement, i.e. the outward transport of calcium, experiments were performed in the absence of extracellular calcium or the presence of deuterium oxide, namely under conditions known to abolish the release of insulin and the concomitant release of $^{45}$calcium induced by insulinotropic agents in isolated islets [3, 7, 8].

## Materials and Methods

The uptake of $^{45}$calcium by isolated islets was measured by a method reported in detail elsewhere [2]. After incubation for 90 min at 37 C in media (1.0 ml) containing $^{45}$calcium (12.5 μC/ml), groups of 75 islets each were submitted to repeated washes in order to remove extracellular $^{45}$calcium. The islets were then transferred, in sub-groups of 5 islets each, in counting vials for measurement of their radioactive content by liquid scintillation. The net uptake of calcium was expressed as pg per islet.

Table 1. *Explanation for symbols used in Fig. 1 to 3*

| | |
|---|---|
| αG | : no glucose |
| G3 | : glucose present (3.0 mg/ml) |
| NCa | : "normal" calcium (2.0 mEq/l) |
| no Ca | : no calcium |
| αCa | : no calcium; EGTA[a] present (1.0 mM) |
| $D_2O$ | : all $H_2O$ replaced by $D_2O$ |
| Dz | : diazoxide present (0.1 or 0.2 mg/ml) |
| S | : gliclazide present (0.025 mg/ml) |

[a] ethyleneglycol-bis-(β-amino-ethyl ether) N,N′-tetra-acetic acid.

The method used for the measurement of $^{45}$calcium efflux has been described in detail elsewhere [3]. Briefly, in each experiment, a group of 200 isolated islets obtained from fed albino rats was incubated for 60 min at 37 C in the presence of glucose (3.0 mg/ml) and $^{45}$calcium (50 to 200 μC/ml). After incubation, the islets were submitted to repeated washes and eventually placed in a small perifusion chamber connected to 2 reservoirs. Each reservoir contained a bicarbonate-buffered medium kept at 37 C and continuously mixed with $O_2$ (95%) and $CO_2$ (5%). In Table 1 are listed the compositions of the various perifusates and the corresponding symbols used in Fig. 1 to 3.

The perifusate was delivered at a constant rate (0.8 to 1.0 ml/min), being derived from the first reservoir during the first 44 or 45 min and from the second reservoir up to the 70th min. From the 31st to 70th min, the effluent was continuously collected in counting vials, over successive periods of one min each. The efflux of $^{45}$calcium (cpm/min) was expressed in per cent of the mean value found within the same experiment during the 5 min preceding the switch from the first to the second perifusate.

## Results

*1. Effect of diazoxide.* Diazoxide is known to inhibit both the accumulation of calcium and the subsequent release of insulin evoked by glucose in isolated islets of Langerhans [2]. At normal calcium concentration, the addition of diazoxide to the perifusate provoked an immediate and pronounced fall in $^{45}$calcium efflux (Fig. 1, left). When compared with previously reported control experiments (see Fig. 5 in Ref. 3), the reduction in $^{45}$calcium efflux was statistically significant ($P < 0.001$) within 2 min after the addition of diazoxide and at all time intervals thereafter. For reasons given elsewhere [3], this reduction in $^{45}$calcium efflux is thought to correspond to the inhibition of glucose-induced insulin release and the concomitant suppression of emiocytosis-associated $^{45}$calcium release.

When the experiments were repeated in the absence of extracellular calcium, i.e. under a condition known to abolish glucose-induced secretion of insulin, the addition of diazoxide provoked an immediate and sustained increase in $^{45}$calcium efflux. Once again, the difference between experimental (Fig. 1, right) and control values (see Fig. 5 in Ref. 3) was significant ($P < 0.05$) within 2 min of exposure to diazoxide. This finding suggests that diazoxide facilitates the outward transport of calcium across the membrane of the beta cell. Such a facilitation could account for the inhibitory effect of diazoxide upon glucose-induced $^{45}$calcium accumulation in isolated islets [2].

Table 2. *Effect of glucose and gliclazide on calcium uptake by islets*

| Glucose (mg/ml) | Gliclazide (mg/ml) | Calcium uptake[a] (pg/islet per 90 min) | |
|---|---|---|---|
| Nil | Nil | 21.3 ± 3.0 | (13) |
| Nil | 0.02 | 90.5 ± 12.6 | (13) |
| 3.0 | Nil | 167.3 ± 16.2 | (13) |
| 3.0 | 0.02 | 187.2 ± 20.4 | (13) |

[a] Mean values (± SEM) are shown together with the number of observations (in parentheses).

*2. Effect of gliclazide.* Table 2 summarizes the effect of gliclazide and glucose on $^{45}$calcium accumulation in isolated islets. Both glucose and gliclazide significantly stimulated the net uptake of $^{45}$calcium, the effect of glucose (3.0 mg/ml) being more marked than that of gliclazide (0.02 mg/ml). At high glucose concentration, gliclazide caused a minor and barely significant further increase ($P < 0.05$ by pairing). These results are comparable to those previously found with glisoxepide or glibenclamide [8]. They suggest that sulfonylureas stimulate the net uptake of calcium by the beta cell.

In the absence of glucose, but at normal calcium concentration, gliclazide, when used at a concentration (0.025 mg/ml) causing maximal insulinotropic response in the rat pancreas [6], provoked an immediate increase in the efflux of $^{45}$calcium. This increase is thought to correspond to the release of $^{45}$calcium concomitant with gliclazide-induced insulin secretion (Fig. 2, left).

In order to abolish this phenomenon, the experiments were performed in the absence of extracellular calcium (Fig. 2, middle) or in calcium-depleted media enriched with $D_2O$ (Fig. 2, right), i.e. under conditions known to suppress the insulinotropic action of sulfonylurea [8]. Under these conditions, gliclazide provoked a transient but highly significant decrease in the efflux of $^{45}$calcium. Thus, the mean lowest value recorded 4 min after the addition of gliclazide averaged $72.1 \pm 2.3$ ($n = 4$) and $80.9 \pm 3.0$ ($n = 3$) per cent, respectively, in the absence of calcium (Fig. 2, middle) and presence of $D_2O$ (Fig. 2, right), as distinct from $95.2 \pm 1.1$ per cent ($n = 4$) in control experiments also performed in the absence of glucose and calcium but without the addition of gliclazide (see Fig. 1, right in Ref. 3). This comparison indicates that gliclazide significantly reduces ($P < 0.005$ or less) the efflux of $^{45}$calcium, suggesting a transient inhibition of outward calcium transport. Such an inhibition was followed by a restoration of $^{45}$calcium efflux to control or even higher values. Although the same pattern of changes in $^{45}$calcium efflux was seen in the absence of calcium (Fig. 2, middle) and presence of $D_2O$ (Fig. 2, right), the secondary elevation was less marked in the presence of $D_2O$. Thus, the maximal mean value for $^{45}$calcium efflux observed at the 58th or 59th min of perifusion averaged $119.8 \pm 4.3$ and $93.0 \pm 1.0$ per cent, respectively in the absence of calcium and presence of $D_2O$.

The effect of gliclazide upon $^{45}$calcium efflux was also investigated in the presence of glucose (3.0 mg/ml). At normal calcium concentration, gliclazide increased $^{45}$calcium efflux (Fig. 3, left). This increase could correspond to an emiocytosis-associated release of $^{45}$calcium, since gliclazide enhances insulin secretion at high glucose concentration [6]. In calcium-depleted media enriched with $D_2O$, the addition of gliclazide provoked an initial fall followed by a secondary rise in $^{45}$calcium efflux (Fig. 3, right), both changes being statistically significant. Indeed, when compared with appropriate control experiments performed at high glucose concentration but in the absence of gliclazide (see Fig. 5, right in Ref. 3), significant differences were observed for both the lowest mean value observed at the 2nd and 3rd min after the addition of gliclazide ($75.0 \pm 1.4$ *vs* $93.2 \pm 1.8$; $P < 0.005$), and the highest mean value observed at the 9th to 13th min after the

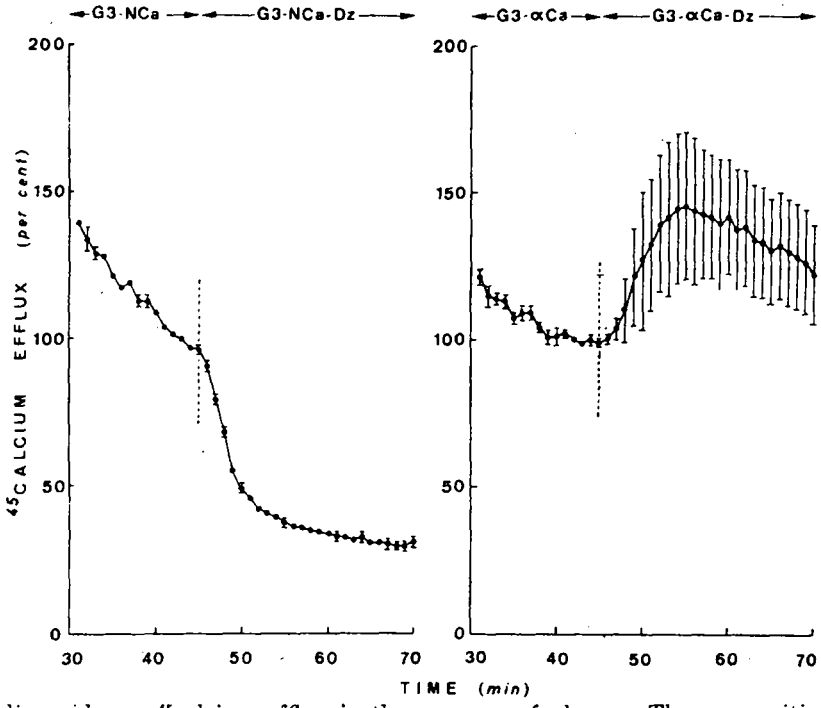


Fig. 1. Effect of diazoxide on $^{45}$calcium efflux in the presence of glucose. The composition of the 2 perifusates administered respectively from 0 to 45 and 46 to 70 min is shown in the upper part of the figure. Mean values (± SEM) for $^{45}$calcium efflux are expressed in per cent (see Materials and Methods) and refer to 2 (left) and 4 (right) individual experiments

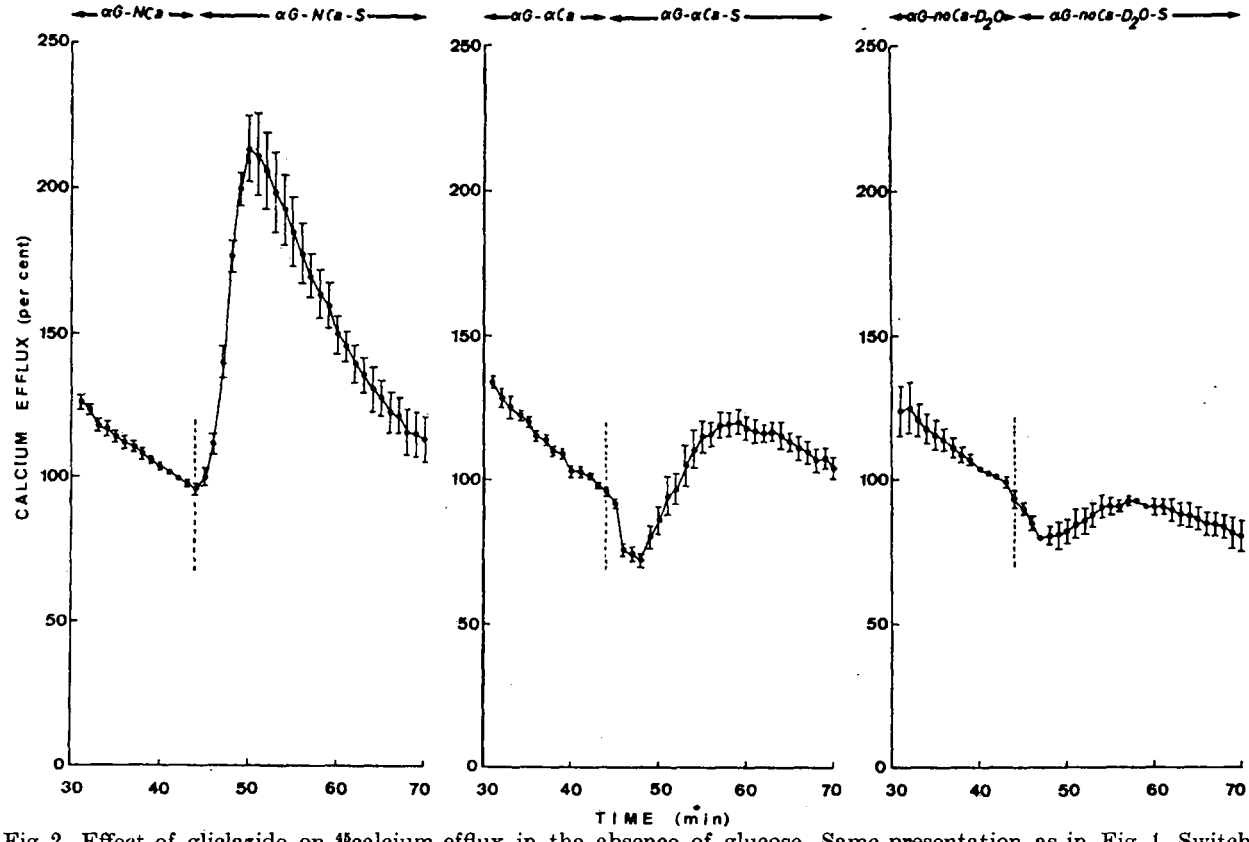


Fig. 2. Effect of gliclazide on $^{45}$calcium efflux in the absence of glucose. Same presentation as in Fig. 1. Switches from the first to the second perifusate was performed after 44 min. Mean values refer respectively to 3 (left), 4 (middle) and 3 (right) individual experiments

addition of gliclazide ($128.0 \pm 13.3$ *vs* $71.8 \pm 3.9$; $P < 0.01$). These findings indicate that, within the limited period of exposure to gliclazide here under study, this sulfonylurea exerts a comparable influence on $^{45}$calcium efflux whether in the absence of glucose or at high glucose concentration.

## Discussion

The results here observed with diazoxide in the presence of glucose are superimposable on those previously obtained when the glucose concentration of the perifusate was suddenly reduced from 3.0 to zero mg/ml [3]. In both cases, the suppression of insulin release at normal calcium concentration was associated with a concomitant reduction of the release of $^{45}$calcium thought to occur at the time and site of emiocytosis. In both cases also, when no secretion of insulin could occur because of the absence of extracellular calcium, there was an immediate and sustained increase in $^{45}$calcium efflux, suggesting a facilitation of the outward transport of calcium across the membrane of the beta cell. In view of this analogy, it is reasonable to assume that diazoxide, by suppressing the inhibitory effect of glucose upon the outward transport of calcium, tends to lower the amount of calcium accumulated in the beta cell and, therefore, to arrest insulin release. This is not to imply that the primary site of action of diazoxide is to inhibit glucose metabolism in the beta cell. Actually, there are reasons to believe that such is not the case. For instance, diazoxide does not affect the stimulant action of glucose on insulin biosynthesis, a process itself depending on the integrity of glucose metabolism in the beta cell [9].

Incidentally, epinephrine, another inhibitor of insulin release, does not provoke a sustained increase in $^{45}$calcium efflux [5]. The discrepancy between the effects of diazoxide and epinephrine on $^{45}$calcium efflux suggests that these two inhibitors of insulin secretion do not act identically in the beta cell. Other arguments in favour of such a lack of identity are (i) the different sensitivity of sulfonylurea-induced insulin release to the respective inhibitory effect of epinephrine and diazoxide [10]; and (ii) the suppression of epinephrine-induced inhibition and maintenance of diazoxide-induced inhibition of insulin release in the presence of alpha-adrenergic blocking agents [11].

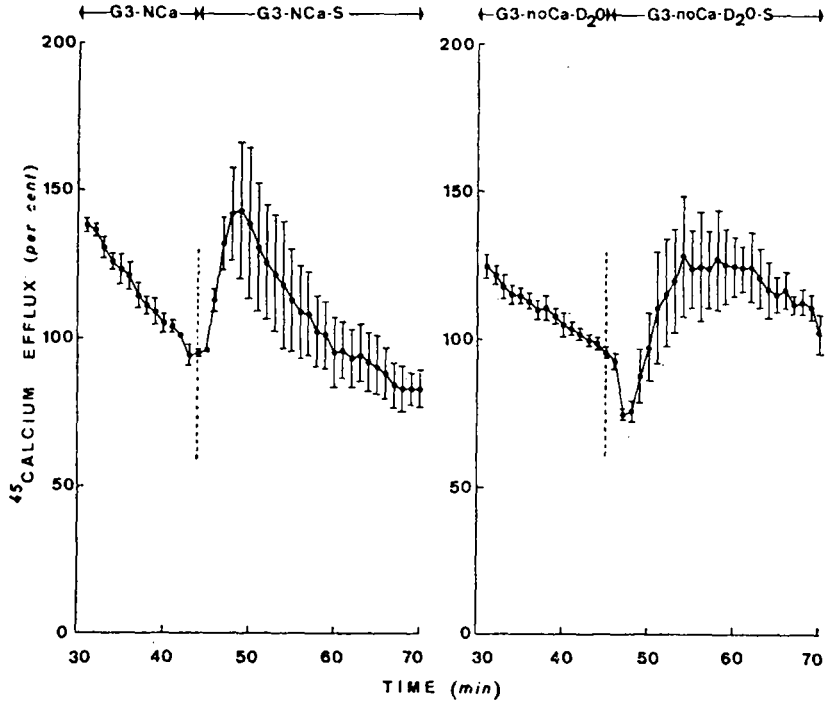


Fig. 3. Effect of gliclazide on $^{45}$calcium efflux in the presence of glucose. Same presentation as in Fig. 1. Switches from the first to the second perifusate were performed after 44 (left) or 45 (right) min. Mean values refer to 3 individual experiments

In the absence of insulin release, the effect of gliclazide upon $^{45}$calcium handling by the beta cell is different from that of the other insulinotropic agents so far investigated in the present system. Thus glucose provokes an immediate and sustained inhibition of outward calcium transport [3]. Theophylline, on the other hand, is thought to cause an intracellular translocation of calcium from the vacuolar system in the cytosol, resulting in an immediate and sustained increase in $^{45}$calcium outflow [4, 5]. In contrast with these monophasic effects, gliclazide provoked, both in the absence and presence of glucose, an early inhibition

of outward calcium transport followed by a significant rebound in calcium outflow.

A possible explanation for the biphasic effect of gliclazide would be that this sulfonylurea exerts a dual effect upon calcium handling by the beta cell. Firstly, it might, by inhibiting the outward transport of calcium, simulate the effect of glucose [3]. Secondly, it might, by causing an intracellular translocation of calcium, mimic the effect of theophylline [5].

Although at present highly speculative, this interpretation of our experimental data is consistent with the concept, outlined in greater detail elsewhere [12], that the insulinotropic action of sulfonylureas displays characteristics of both glucose-simulation and glucose-potentiation. Briefly, it was shown that sulfonylureas both lower the "Km" (glucose-simulating effect) and increase, at least transiently, the "Vmax" (glucose-potentiating effect) of the sigmoidal curve relating the rate of insulin secretion to the glucose concentration of the fluid bathing the beta cell [12]. The glucose-simulating action of gliclazide would account not only for the inhibition of outward calcium transport, but also for the accumulation of $^{45}$calcium in isolated islets. Incidentally, because of the postulated dual effect of sulfonylurea, the present data do not allow us to assess the duration of the glucose-simulating effect of gliclazide. In previous work, we have obtained data to suggest that the stimulatory effect of certain sulfonylureas upon $^{45}$calcium accumulation in the beta cell and their subsequent insulinotropic action fades out more rapidly at high than at low glucose concentration [8, 10]. This might explain why the gliclazide-induced increment in the amount of $^{45}$calcium recovered in the islets at the 90th min of incubation was more marked in the absence of glucose than at high glucose concentration (Table 2), despite a comparable immediate inhibitory effect on $^{45}$calcium efflux at these two glucose levels (Fig. 2, middle; Fig. 3, right). The second postulated mode of action of gliclazide, namely its theophylline-like or glucose-potentiating action, is consistent with the recent demonstration that sulfonylureas inhibit cyclic-3′,5′-AMP phosphodiesterase activity in insular tissue [13, 14]. From our data, we would be led to suggest that the glucose-simulating action of gliclazide upon $^{45}$calcium efflux is masked by its theophylline-like effect after approximately 3 to 4 min of exposure to this sulfonylurea. It is remarkable that such a sequence of changes in $^{45}$calcium efflux is chronologically identical to the sequence of changes in electrical activity recently reported by Pace and Price [15] in islets exposed to tolbutamide.

In conclusion, the present data add further support to our working hypothesis that a variety of agents known to affect insulin secretion do so by causing immediate changes in the handling of calcium by the beta cell.

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