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Calcium Antagonists and Islet Function: VII. Effect of Calcium Deprivation

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Summary. The role of extracellular Ca^{2+} in the regulation of islet function is investigated. Decreasing extracellular Ca^{2+} concentrations cause a dose-related inhibition of glucose-induced insulin release. Whereas the efflux of ^{45}Ca from perifused islets is transiently increased on exposure to Ca^{2+}-deprived media, it is unaffected by a partial lowering of the extracellular Ca^{2+} concentration. Under the latter condition, therefore, the observed reduction in the size of the islets' exchangeable calcium pool(s) appears to be due to reduced Ca^{2+} entry. The proper effect of glucose on Ca handling by the islets is apparently not affected by a lowering in the extracellular Ca^{2+} concentration. Nevertheless, in islets exposed to glucose and incubated in Ca^{2+}-deprived media, glucose uptake and oxidation and lactate output are decreased, whereas the islet ATP level is increased, as if extracellular Ca^{2+} shortage were to affect not only the cellular pool of Ca regulating insulin release, but also energy-consuming processes possibly located at the cell membrane.

Whereas the omission of extracellular Ca^{2+} has long been known to abolish insulin release [6, 27], it was only recently reported that Ca^{2+} *per se* is able to stimulate the secretion of insulin in the absence of any other insulinotropic agent [5]. The secretory response to Ca^{2+} is abolished by verapamil, which is thought to inhibit Ca^{2+} inward transport in the B-cell [12]. These findings afford direct support to the idea that Ca accumulation in some critical site of the B-cell is sufficient to trigger insulin release [13]. Recent reports on the effect of ionophores upon insulin release also support such a view [3, 10, 31]. Since, in addition, all insulinotropic agents so far investigated for this purpose – including hexoses, glyceraldehyde, amino acids, cyclic AMP, theophylline, K^+ and sulfonylureas – were found to affect Ca handling by isolated islets [1, 2, 14, 17, 20, 22, 25, 26], it is reasonable to assume that these agents

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also exert their stimulant action upon insulin release by eventually causing Ca accumulation in the B-cell. In the present report, we have investigated to what extent the effect of glucose upon Ca handling and other parameters of islet function may itself depend on the availability of extracellular Ca^{2+}.

Materials and Methods

All experiments were performed with pancreatic tissue removed from fed rats. The methods used for the measurement of insulin release by the isolated perfused pancreas [30], and insulin secretion [15], glucose uptake [21], lactate production [17] and glucose oxidation [24] in isolated islets are all described in previous publications.

Two methods were used for the measurement of ^{45}Ca net uptake by the islets. The first procedure, used in most of the present experiments, is described [26] and its significance discussed [18] in detail elsewhere. Briefly, the net uptake of ^{45}Ca is judged from the radioactive content of the islets after 90 min incubation in the presence of $^{45}Ca^{2+}$ and $^{40}Ca^{2+}$, followed by a washing procedure designed to remove the extracellular $^{45}Ca^{2+}$. The total length of the washing procedure performed at room temperature never exceeds 40 min. Because the amount of ^{45}Ca released by the islets during the last washes is proportional to their final radioactive content [20, 26], the latter value is assumed to be itself proportional to the true amount of ^{45}Ca accumulated in the islets at the 90th min of incubation. In order to validate such an assumption, a limited series of measurements were performed with a second technique which does not involve any washing procedure. Groups of 10 islets each were transferred into plastic polythene microcentrifuge tubes and incubated in a small volume (75 µl) of the usual bicarbonate-buffered medium containing $^{45}Ca^{2+}$ (20 µCi/ml) and D-(1-^{3}H(N)-mannitol (5.6 mM; 20 µCi/ml). Ten min prior to the completion of the incubation period, silicon oil (0.1 ml; Versilube F50; General Electric, Bergen-op-Zoom, Netherlands) was carefully layered on top of the incubation medium. At the end of the incubation period, the tubes were centrifuged for 10 sec at $11{,}500 \times g$ (Beckman Microfuge, Model 152). Each tube was inspected to ensure that the islets formed a solid pellet in the tip of the tube. The lowest two mm of the tube containing the islet pellet and some surrounding oil were removed with a scalpel, transferred to a scintillation vial containing 1 ml of a solution of 2 mM EGTA (pH 7.0), mixed with 10 ml of Instagel (Packard, Downers Grove, Illinois), subjected to sonification, and eventually examined for their ^{45}Ca and ^{3}H content. The amount of ^{45}Ca which was present in the islets, in excess of that which could be accounted for by the ^{3}H-mannitol apparent space of distribution, was taken as the net uptake of ^{45}Ca and expressed as pg/islet. The absolute values obtained by the oil separation procedure were approximately twice as high as those obtained by the washing procedure, a finding consistent with the fact that, in the latter procedure, each of the five successive washes removes approximately 10–15% of the final radioactive content of the islets [26]. However, the two methods gave essentially the same results as far as the relative influence of glucose, Ca^{2+}, and incubation time upon $^{45}Ca^{2}$ net uptake is concerned (Fig. 1).

The efflux of ^{45}Ca is examined after prelabeling of the islets over 60-min incubation at 37 °C in the presence of glucose (16.7 mM), $^{45}Ca^{2+}$ and $^{40}Ca^{2+}$ (total Ca^{2+} concentration 2.24 meq/liter) and washing of the islets (*see above*). The islets are then placed in a perifusion chamber and the effluent radioactivity monitored from the 30th min of perifusion onwards. Obviously, the effluent radioactivity is not necessarily indicative of the efflux of ^{40}Ca. At least, it is representative of the release of ^{45}Ca. Therefore, any situation affecting ^{45}Ca net uptake but not ^{45}Ca efflux is likely to exert its effect upon the net uptake

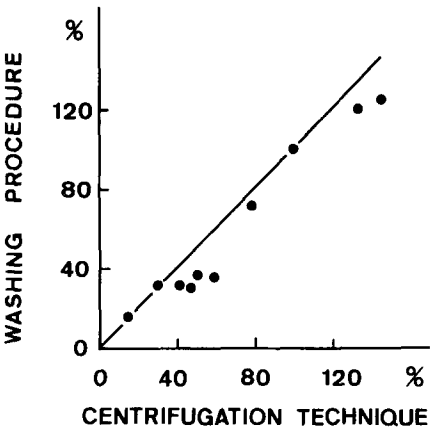

Fig. 1. Effect of glucose, extracellular Ca^{2+} and incubation length upon ^{45}Ca net uptake by isolated islets, using either the washing procedure or the oil separation technique. The ten experimental conditions under study and the mode of expression for results are identical to those outlined in Table 1. The data relative to the washing procedure are derived from the curves illustrated in Figs. 2 (lower panel) and 6 (left panel)

of ^{45}Ca through alteration of the rate of $^{45}Ca^{2+}$ entry in the islet cells. It is here assumed that such a rate of $^{45}Ca^{2+}$ entry is representative of that of $^{40}Ca^{2+}$ entry.

For the measurement of the ATP content of the islets, groups of 15 islets or more were incubated for 90 min in the usual medium. After removal of the incubation medium, the islets were resuspended in 1.2 ml of iced and desionized H_2O containing $HClO_4$ 25 mM, and submitted to sonification. After centrifugation, the ATP content of the supernatant solution (1.0 ml) was assayed extemporaneously by the luciferin-luciferase method [29]. The bioluminescence was linearly related to the ATP standards (25 to 500 pmoles per tube).

Results

Insulin Release

Fig. 2 illustrates the influence of increasing Ca^{2+} concentrations (0 to 4 meq/liter) upon insulin release evoked by glucose (16.7 mM) in the isolated islets. Over the present range of concentrations, a greater than fivefold increase in insulin output was noticed. In the isolated perfused pancreas, the administration of a perfusate enriched with EGTA during the second phase of the secretory response to glucose (16.7 mM) caused a rapid and reversible inhibition of insulin release to basal values (Fig. 3).

Calcium Efflux

Most of the experiments designed to study the influence of Ca^{2+} upon its rate of efflux from perifused islets were performed in the absence

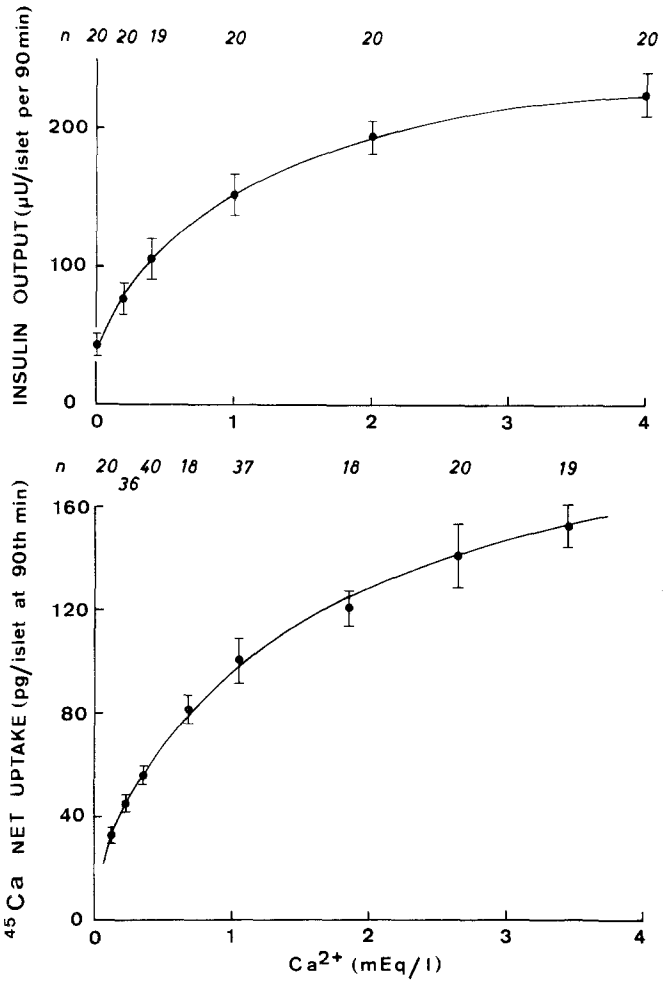

Fig. 2. Mean values (±SEM) for insulin output and Ca net uptake (washing procedure) by isolated islets incubated at increasing extracellular Ca^{2+} concentrations are shown together with the number of individual determinations (*n*)

of glucose to avoid interference of the so-called exocytotic release of ^{45}Ca usually associated with stimulation of insulin secretion [14].

When the prelabelled islets were first perifused at a normal extracellular Ca^{2+} concentration (2 meq/liter), and then suddenly exposed to a medium deprived of Ca^{2+} and enriched with EGTA, a sudden but short-lived burst of radioactivity appeared in the effluent (Fig. 4, upper panel). When the perifusate administered from the 44th min onwards was also deprived of Ca^{2+} but contained no EGTA, the transient release of ^{45}Ca,

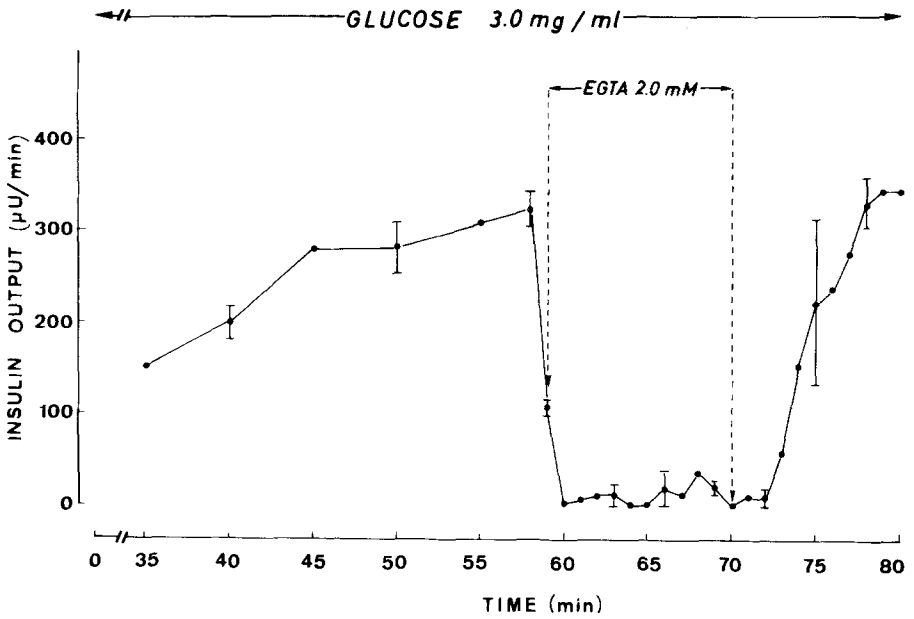

Fig. 3. Mean values (±SEM) for insulin output by the isolated perfused rat pancreas refer to two individual experiments. Glucose (16.7 mM) and Ca^{2+} (2 meq/liter) were present throughout the experiment. EGTA was added from the 60th to 70th min

which could be due to the depletion of a small Ca pool, occurred less abruptly (Fig. 5, upper panel). In both situations, however, the integrated amount of effluent radioactivity (min 45 to 70) was almost identical, averaging 25.2 and 27.3 times the appropriate control value (44th min) in the presence and absence of EGTA, respectively.

Glucose (16.7 mM) was apparently able to prevent the depletion phenomenon (Fig. 5, lower panel). Indeed, in the presence of glucose, the omission of extracellular Ca^{2+} failed to cause the transient increase in effluent radioactivity and, instead provoked a rapid fall in ^{45}Ca release. Such a behavior, which is superimposable to that seen at normal Ca^{2+} concentration (2 meq/liter) when glucose itself is removed from the perifusate [14], probably corresponds to the arrest of insulin release with a concomitant suppression of exocytosis-associated ^{45}Ca release. The depletion phenomenon also failed to occur when, in the absence of glucose, the extracellular Ca^{2+} concentration was lowered to half its normal value (Fig. 4, lower panel). The latter finding indicates that a partial lowering of the extracellular Ca^{2+} concentration is not associated with any obvious facilitation of ^{45}Ca exit.

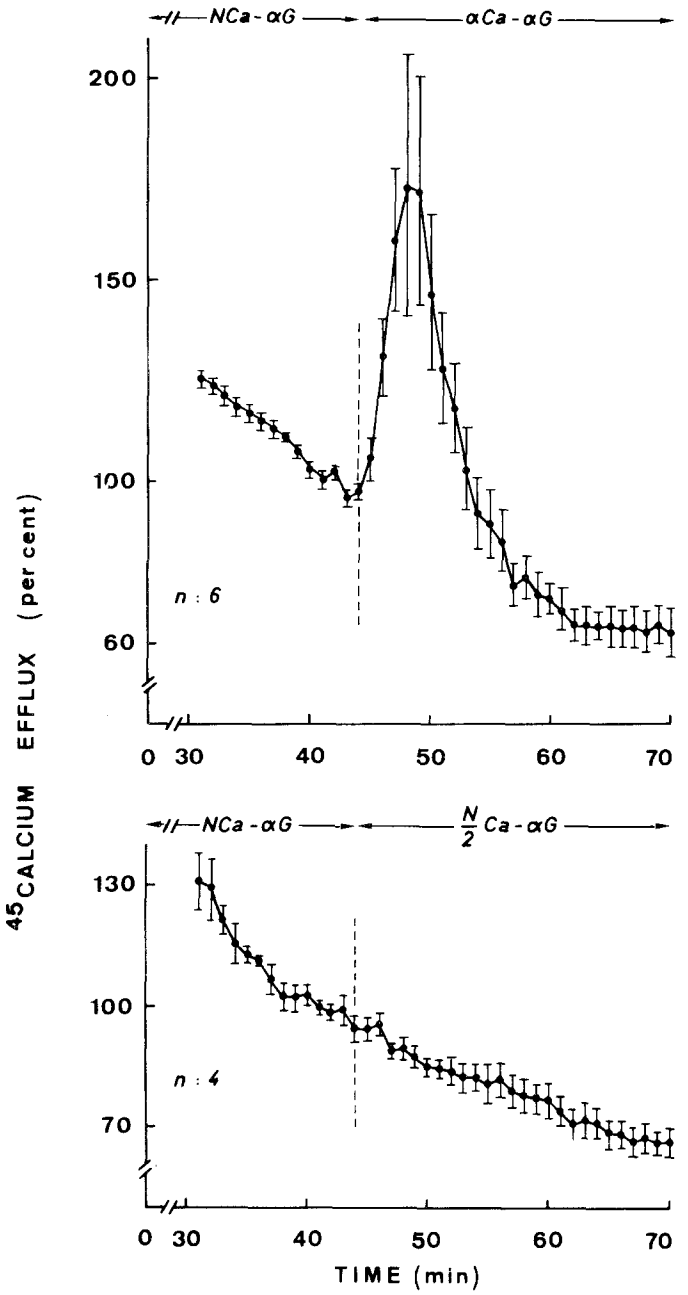

Fig. 4. The efflux of ^{45}Ca from perifused islets is expressed in percent of the mean control value found within each experiment between the 40th and 44th min. The glucose-free (αG) perifusates either contained Ca^{2+} in normal (2 meq/liter; NCa) or half-normal (N/2) concentration, or were deprived of Ca^{2+} and enriched with EGTA (1.0 mM; αCa). The composition of the perifusate was changed at the time shown by the dotted line. Mean values (±SEM) are shown together with the number of individual observations (*n*)

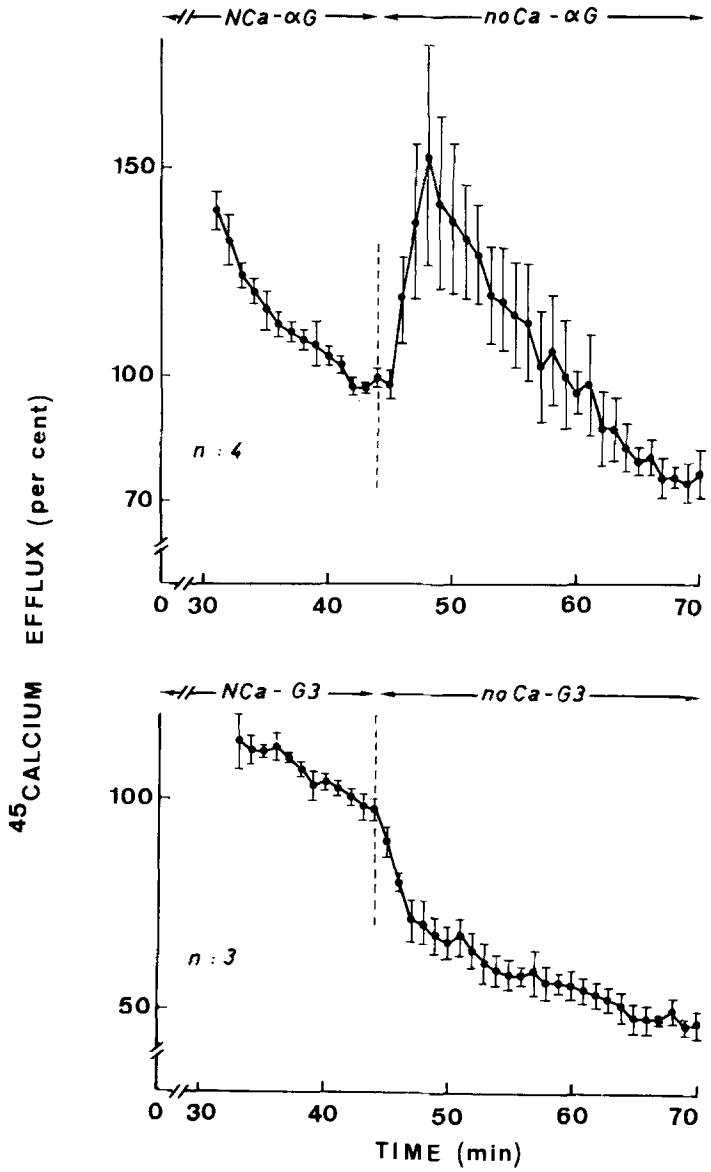

Fig. 5. Same presentation as in Fig. 4. The experiments were performed in the absence (αG) or presence of glucose (16.7 mM; G3), and at normal Ca^{2+} concentration (2meq/liter; NCa) or in the absence of extracellular Ca^{2+} (no Ca)

Calcium Net Uptake

The curves relating both glucose-induced insulin release and ^{45}Ca net uptake by the islets to the extracellular Ca^{2+} concentration ran grossly in parallel fashion (Fig. 2). The data on ^{45}Ca net uptake were obtained

either by using constant specific activity for $^{45}Ca^{2+}$ in the incubation medium (low extracellular Ca^{2+} concentrations) or by diluting a constant amount of $^{45}Ca^{2+}$ with increasing amounts of $^{40}Ca^{2+}$ (high extracellular Ca^{2+} concentration). The validity of this procedure was assessed by the fact that, at a given Ca^{2+} concentration (0.36 meq/liter), the estimation of Ca net uptake was not significantly affected by the degree of isotopic dilution, averaging 58.9 ± 4.4 and 53.6 ± 6.1 pg/islet ($n = 20$ in each case) in media containing $^{45}Ca^{2+}$ at theoretical specific activities of 20.0 and 6.7 μCi/μg, respectively. The influence of extracellular Ca^{2+} upon glucose-induced ^{45}Ca net uptake was confirmed with the oil separate procedure (Table 1), the values averaging 65 ± 8, 229 ± 27 and 332 ± 63 pg/islet ($n = 15$ or 16) at the 90th min of incubation in the presence of Ca^{2+} 0.2, 2.0 and 4.0 meq/liter, respectively.

Incidentally, in considering the influence of extracellular Ca^{2+} upon ^{45}Ca net uptake, the time factor is apparently not of critical importance. Indeed, whether at normal (2.0 meq/liter) or low (0.32 meq/liter) Ca^{2+} concentration, the values for glucose-induced ^{45}Ca net uptake continuously increase over 135 min incubation, tending towards saturation at almost the same rate (Fig. 6, right panel). This continuous increase contrasts with the behavior found in the absence of glucose and at normal Ca^{2+} concentration. Under the latter condition, the values for ^{45}Ca net uptake reach much more rapidly equilibrium, no significant difference ($p > 0.6$) being noted between the measurements performed at the 45th and 135th min, respectively. These contrasting behaviors are compatible

Table 1. Effect of glucose, extracellular calcium and incubation time upon the net uptake of ^{45}calcium by isolated islets, as measured by the oil separation procedure[a]

Time (min)	Calcium (meq/liter)	Glucose	
		Nil	16.7 mM
20	2.0	13.1 ± 1.5 (6)	59.5 ± 13.9 (10)
45	2.0	41.3 ± 11.4 (11)	78.4 ± 6.6 (13)
90	0.2		28.5 ± 3.7 (15)
90	2.0	46.1 ± 9.4 (23)	100.0
90	4.0		145.1 ± 27.4 (15)
135	2.0	49.1 ± 6.0 (13)	131.5 ± 20.8 (13)

[a] All data are expressed relative to the appropriate mean control value found within the same series of experiments after 90 min incubation in the presence of Ca^{2+} (2 meq/liter) and glucose (16.7 mM). Such a control value averages 316 ± 34 pg/islet ($n = 51$). Mean values (± SEM) are shown together with the number of individual observations (in parentheses).

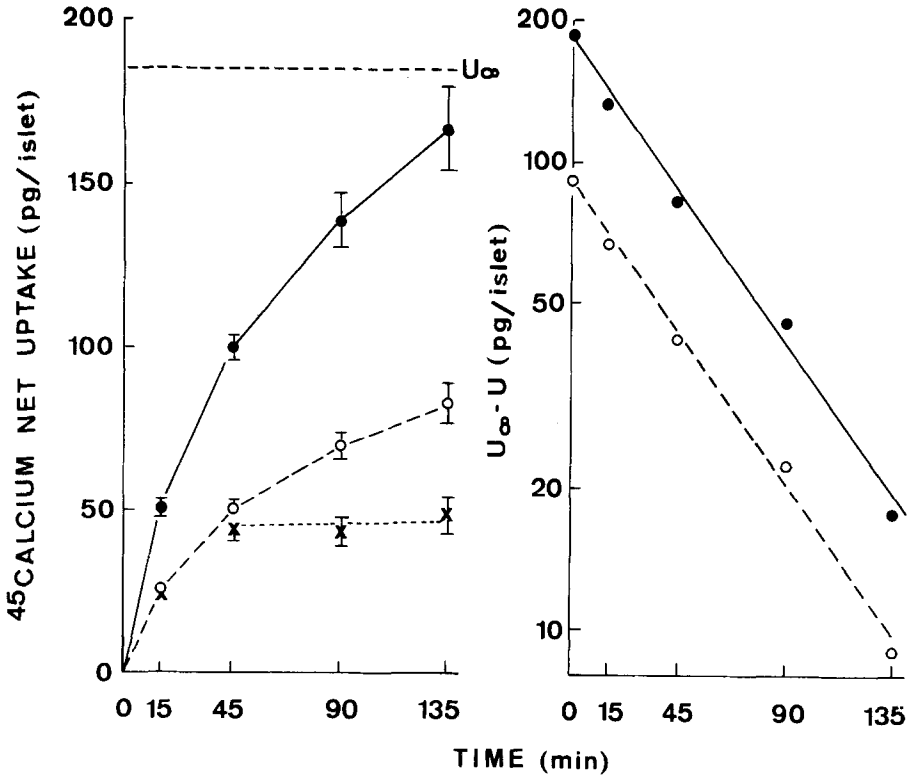

Fig. 6. In the left panel, the data refer to the net uptake of ^{45}Ca (washing procedure) at normal (2 meq/liter; closed circles) or low (0.32 meq/liter; open circles) Ca^{2+} concentration in the presence of glucose (16.7 mM). The data obtained at normal Ca^{2+} concentration in the absence of glucose (crosses) are shown for purpose of comparison. Each value represents the mean (±SEM) of 9 to 37 individual measurements. Also shown is the equilibrium value (U_∞) for the data found in the presence of glucose at the normal Ca^{2+} level. In the right panel, the difference between equilibrium and experimental value (U_∞-U) are plotted in semilogarithmic coordinates

with the view that glucose provokes a progressive enrichment of the B-cell calcium pool(s) both at normal or low Ca^{2+} concentration.

The influence of the length of incubation with $^{45}Ca^{2+}$ upon its accumulation in the islets, as depicted in Fig. 6, was confirmed in experiments carried out using the oil separation procedure (Table 1). With the latter technique, no significant increase in ^{45}Ca net uptake occurred between the 45th and 135th min of incubation in the absence of glucose, at which time the uptake averaged, respectively, 89.5 ± 24.7 and $106.5 \pm 13.1\%$ of the mean value found at the 90th min of incubation ($n = 11$ to 13). Such a situation contrasts with that found in the presence of glucose (16.7 mM), in which case the net uptake of ^{45} Ca, relative to the

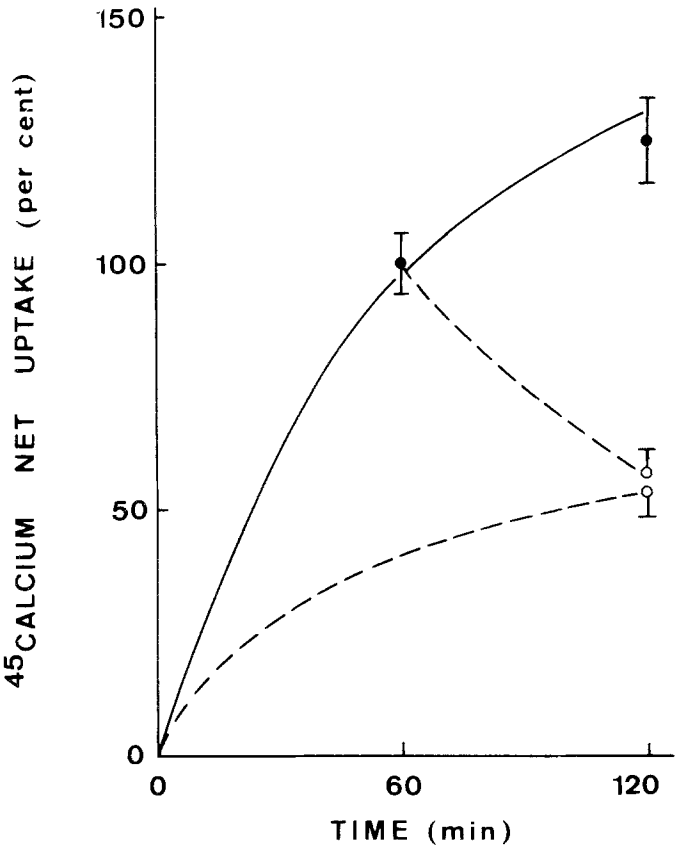

Fig. 7. Reversibility of the stimulant action of glucose upon ^{45}Ca net uptake (washing procedure). Islets were incubated either in the presence (●—●) or absence (○—○) of glucose (16.7 mM). Mean values (±SEM) refer to 12–38 individual measurements and are expressed in percent of the mean control value found within the same experiment after 60 min exposure to glucose. Such a control value averaged 170.8±11.3 pg/islet ($n=38$)

mean value measured at the 90th min, significantly increased ($p<0.02$) from 78.4±6.6% after 45 min incubation to 131.5±20.8% at the 135th min ($n=10$ to 13).

Further support in favor of the view that the effect of glucose on ^{45}Ca net uptake corresponds to a true increase in the size of the calcium pool(s) was searched by examining the reversibility of the glucose-induced changes in ^{45}Ca net uptake (Fig. 7). Islets were first incubated for 60 min in the presence of glucose (16.7 mM), $^{40}Ca^{2+}$ (2.0 meq/liter) and $^{45}Ca^{2+}$ (0.4 meq/liter). Thereafter, the islets were transferred to a fresh medium deprived of glucose but still containing the same amount of both $^{40}Ca^{2+}$ and $^{45}Ca^{2+}$. At the end of the second incubation period (120th min), the radioactive content of the islets only represented 57.6±4.2% ($n=37$)

Table 2. Mean values (±SEM) for ^{45}Ca net uptake (pg/islet) by isolated islets incubated for 90 min at two glucose and two extracellular Ca^{2+} concentrations are shown together with the number of individual determinations (in parentheses)

Ca^{2+} (meq/liter)	Glucose	
	Nil	16.7 mM
0.24	10.6 ± 1.6 (20)	46.0 ± 2.7 (44)
2.00	36.8 ± 3.9 (20)	117.3 ± 5.6 (26)

[a] The net uptake of ^{45}Ca was measured by the washing procedure.

of the mean control value found, in paired groups of islets, at the end of the first period of incubation of 60 min in the presence of glucose (100.0 ± 6.6%, $n = 38$). These data clearly indicate that the net uptake of ^{45}Ca, as measured by the present technique, is not merely the reflection of a process of isotopic equilibration. Indeed, the fall in the radioactive content of the islets, as seen between the 60th and 120th min, occurred despite unchanged radioactivity of the incubation medium. The magnitude of the fall in radioactivity of the islets observed on exposure to the glucose-free medium during the second hour of incubation suggested that the exchangeable calcium pool(s) had eventually reached at the 120th min the same size as that seen after a 120 min incubation period performed throughout in the absence of glucose.

The stimulant action of glucose upon Ca net uptake by the islets, relative to the corresponding basal value found in the absence of glucose, was not less marked at low than at normal Ca^{2+} concentration (Table 2).

Glucose Metabolism

The metabolism of glucose does not occur at a normal rate in islets exposed to a Ca^{2+}-depleted medium (Table 3). In Table 3, the data for lactate output and glucose oxidation obtained in absence of Ca^{2+} and presence of EGTA (1.0 mM) are compared to those found in the presence of mannoheptulose (14.3 mM), a known inhibitor of glucose phosphorylation in the islets [19]. Comparison between experimental and control values indicated that the reduction in glucose metabolism averaged −20.8 ± 5.3% ($p < 0.01$) in the absence of calcium and presence of EGTA as distinct from −54.6 ± 4.6% ($p < 0.001$) in the presence of mannoheptulose. The same degree of inhibition was observed at a noninsulinotropic glucose concentration (4.2 mM) than at a much higher glucose level

Table 3. Mean values (±SEM) for glucose oxidation and uptake, and lactate output in isolated islets are shown together with the number of individual observations (in parentheses)[a]

Ca^{2+} (meq/liter)	2.0	–	–	2.0	2.0
Mg^{2+} (meq/liter)	2.0	2.0	2.0	20.0	2.0
EGTA (mM)	–	1.0	–	–	–
Mannoheptulose(mM)	–	–	–	–	14.3

Glucose (mM)	Metabolic parameter	Control values (pmole/islet per 60 min	*Experimental values (% of control)*			
4.2	Glucose oxidation	8± 2 (12)	*79.5±11.9 (12)*			*50.6±11.9 (12)*
4.2	Lactate output	36± 2 (6)	*79.8± 7.3 (6)*			*50.5± 7.3 (6)*
16.7	Glucose oxidation	19± 5 (11)	*82.8±12.9 (11)*			*37.4± 7.2 (12)*
16.7	Lactate output	68± 4 (13)	*80.6± 8.0 (6)*	*67.5±7.3 (8)*	*69.2±14.2 (6)*	*43.1± 3.5 (6)*
16.7	Glucose uptake	112±13 (8)	*65.8± 7.4 (4)*		*54.0± 5.3 (3)*	*49.3±16.9 (4)*

[a] Control values are expressed as pmoles of glucose residue/islet per 60 min. Experimental values are expressed in percent of the mean (glucose oxidation and lactate output) or paired (glucose uptake) control value found within the same experiment.

Table 4. Mean values (±SEM) for ATP content in isolated islets exposed for 90 min to glucose (16.7 mM) are shown together with the number of individual determinations (in parenthesis) and the statistical significance between control (first line) and experimental values obtained within the same experiment(s)

Ca^{2+} (meq/liter)	EGTA (mM)	ATP content (pmoles/islet at 90th min)	
2.0	–	4.5±0.5 (23)	5.7±0.7 (17)
–	–	7.7±1.0 (24)[a]	
–	1.0		8.9±0.8 (17)[a]

[a] $p<0.01$.

(16.7 mM). The reduction in glucose metabolism found in Ca^{2+}-depleted media is unlikely to be due to EGTA. Thus, glucose uptake was also reduced when an abnormally high extracellular Mg^{2+} concentration was used to inhibit Ca^{2+} entry in the B-cell [16]. Moreover, the output of lactate was also significantly depressed either in the absence of both Ca^{2+} and EGTA or in the presence of an excess of Mg^{2+} (Table 3).

A possible explanation for the reduction in glucose metabolism was sought by measuring the islets content in ATP which, in high concentration, is known to inhibit the activity of islets phosphofructokinase [23]. After 90 min incubation in Ca^{2+}-depleted media, whether in the absence or presence of EGTA, the ATP content of islets exposed to glucose (16.7 mM) was significantly higher than that found at normal Ca^{2+} concentration (Table 4).

Discussion

The rapid arrest of glucose-induced insulin release upon chelation of extracellular Ca^{2+} (Fig. 3) confirms prior observations [4, 9, 11] and suggests that a continuous supply of extracellular Ca^{2+} is required for either (i) the integrity of secretory event(s) located at the plasma membrane and directly influenced by the level of extracellular Ca^{2+}, or (ii) the maintenance of a critical cellular pool of Ca controlling insulin release and characterized by a high fractional turn-over rate. In our view, the second hypothesis is more likely, mainly because the process of insulin secretion is also responsive to agents affecting the intracellular movements of Ca [18]. Within the framework of this second hypothesis, the present work aimed at a more careful assessment of the influence of extracellular Ca^{2+} upon Ca handling by the B-cell.

The short-lived burst of ^{45}Ca efflux which occurs on exposure of the islets to media deprived of Ca^{2+} (Figs. 4 and 5, upper panels) is suggestive of the sudden depletion of a limited and possibly membrane-associated Ca pool. It amounted to no more than 17.7 (in the presence of EGTA) and 18.9% (in the absence of EGTA) of the integrated effluent radioactivity otherwise collected during the late period of perifusion (45th to 70th min). This postulated depletion, which was only detected in the absence of glucose (*see* Fig. 5), may account for a change in plasma membrane permeability, so that extracellular Ca^{2+}, when reintroduced in the system, can enter the B-cell in sufficient amount to trigger insulin release [5].

When extracellular Ca^{2+} is partially diminished but not completely suppressed, no detectable change in ^{45}Ca efflux occurs (Fig. 4, lower panel). The reduction in size of the calcium exchangeable pool(s) seen under this condition (Fig. 2) is likely, therefore, to be due mainly to a reduction in the rate of Ca entry in the B-cell. Our experimental data suggest that such an inward transport of Ca in islet cells is indeed mediated through a finite number of carriers (Fig. 2). Within the

range of Ca^{2+} concentrations here examined, a reduction in the extracellular Ca^{2+} level apparently does not interfere with the proper effect of glucose on Ca^{2+} handling. Indeed, the glucose-induced inhibition of Ca outward transport across the B-cell membrane [14] and subsequent increase in the pool of exchangeable calcium (Table 2) are both unaffected at low or null extracellular Ca^{2+} concentrations.

Although glucose-sensitive events such as stimulation of Ca net uptake and proinsulin biosynthesis [28] are not impaired in Ca^{2+}-depleted media, the metabolism of glucose is moderately but significantly reduced in the absence of extracellular Ca^{2+}. Hellman *et al.* [8] reported that glucose oxidation is diminished and the level of fructose 1,6-diphosphate increased in islets exposed to Ca^{2+}-deprived media. The reduction in glucose uptake, lactate production and glucose oxidation here observed cannot be attributed solely to the arrest of the insulin secretory process since it also took place at a noninsulinotropic glucose concentration (Table 3). Moreover, when insulin release is abolished by use of Ca^{2+}-antagonists such as verapamil, no reduction in glucose metabolism is observed [12].

As an alternative and provocative hypothesis, it could be postulated that the reduction in glucose metabolism is due to the failure of extracellular Ca^{2+} to reach an appropriate system located at the plasma membrane, rather than being due to the cellular depletion in Ca. This concept is compatible with the fact that, in high concentration, Mg^{2+}, which might compete with Ca^{2+} for the postulated membrane site, mimics the effects of Ca^{2+} omission (Table 3). It is also consistent with the idea that a major fraction of the ATP generated in the B-cell is used for the maintenance of correct ionic gradients across the plasma membrane [7]. For instance, a reduction in membrane-associated ATPase activity could, by increasing the ATP concentration, lead to a reduced glycolytic flux. Incidentally, the ATP concentrations here measured (1 to 3 mM, assuming even intracellular distribution) indeed coincide with those causing a dose-related inhibition of islets phosphofructokinase activity [23].

In conclusion, Ca^{2+} may exert a multiple control upon B-cell function, a sufficient supply of extracellular Ca^{2+} being (i) usually required for the maintenance of the Ca cellular pool mediating insulin release and (ii) apparently involved in the regulation of energy-consuming processes possibly located at the cell membrane.

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