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Regulation of Calcium Fluxes in Rat Pancreatic Islets: Calcium Extrusion by Sodium-Calcium Countertransport*

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Summary. The mechanisms by which glucose regulates calcium fluxes in pancreatic endocrine cells were investigated by monitoring the efflux of ^{45}Ca from prelabeled and perifused rat pancreatic islets. In the absence of both extracellular calcium and glucose, partial or total removal of extracellular sodium decreases the efflux of ^{45}Ca from prelabeled islets. Glucose also reduces the efflux of ^{45}Ca from islets perifused in the absence of extracellular calcium. This inhibitory effect of glucose on ^{45}Ca efflux is decreased by half when the extracellular concentration of sodium is lowered to 24 mM. In the absence of extracellular calcium but presence of glucose, partial or even total removal of extracellular sodium fails to decrease the efflux of ^{45}Ca. At normal extracellular calcium concentration (1 mM) partial removal of extracellular sodium dramatically increases ^{45}Ca efflux from pancreatic islets. This increase in ^{45}Ca efflux is partially but not totally suppressed by either 16.7 mM glucose or cobalt. It is totally suppressed by 4.4 mM glucose or by the combination of 16.7 mM glucose and cobalt. At normal extracellular calcium concentration, glucose initially reduces and subsequently increases ^{45}Ca efflux. The initial fall is unaffected by tetrodotoxin but decreased by 50% at low extracellular sodium concentration (24 mM). The present results suggest the existence in pancreatic endocrine cells of a glucose-sensitive process of sodium-calcium countertransport. By inhibiting such a process, glucose may decrease the efflux of calcium from islet cells. The effect of glucose is not mediated by an increase in intracellular sodium concentration. It could contribute to the intracellular accumulation of calcium which is thought to trigger insulin release.

Key words: Sodium-calcium countertransport, insulin release – pancreatic islet cells – cobalt.

* This paper is the IVth in a series.

The process of glucose-induced insulin release coincides with and might be triggered by an increase in the intracellular concentration of calcium [24]. Electrophysiological studies suggest that glucose stimulates calcium entry into the B-cell by opening calcium channels [9]. Studies of the unidirectional efflux of ^{45}Ca from prelabeled and perifused islets suggests that the effect of glucose on calcium fluxes could be more complex. Thus, a sudden increase in the glucose concentration of the perifusate from zero to 16.7 mM provokes an initial fall followed 3 to 4 min later by a dramatic increase in ^{45}Ca efflux [7, 18, 19, 25]. The latter rise in efflux apparently reflects an increase in the rate of ^{40}Ca influx through opened voltage-sensitive calcium channels [17] and corresponds to a calcium-calcium exchange mechanism [12, 17, 18, 22]. Glucose may also inhibit calcium efflux from the islet cells. This inhibitory effect, which is responsible for the initial fall in ^{45}Ca efflux, represents a sustained phenomenon operative throughout the period of exposure to glucose [19], but is masked by the secondary rise in effluent radioactivity [19, 22]. The inhibitory effect of glucose corresponds to a true reduction in ^{40}Ca efflux [22] and, as such, could be due to an inhibition of calcium outward transport across the B-cell plasma membrane.

The mechanism by which calcium is actively extruded from the B-cell remains unknown. Although a calcium-activated ATPase activity was demonstrated in subcellular fractions of mouse pancreatic islets [11] and in homogenates of rat pancreatic islets [23], no evidence is available to suggest that such an ATPase participates in the active outward transport of calcium across the B-cell plasma membrane. Another system able to eject calcium from cells against its electrochemical gradient is the sodium-calcium countertransport, which has been described in a large variety of tissues (*see* [1, 5] for review). In this system, the movement of sodium into the cells down its elec-

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trochemical gradient provides the energy for uphill calcium extrusion. The existence of such a process exchanging sodium for calcium has been postulated in the pancreatic B-cell [30]. For instance, it was suggested that the initial inhibitory effect of glucose on ^{45}Ca efflux from pancreatic islets could be due to inhibition of such a sodium-calcium countertransport [10].

The aim of the present study was to obtain further evidence for the existence in pancreatic endocrine cells of a process of sodium-calcium countertransport and its regulation by glucose.

Materials and Methods

Incubation, washing, and perifusion media. The media used for incubating, washing, or perifusing the islets consisted of a Krebs-Ringer bicarbonate-buffered solution supplemented with 0.5% (wt/vol) dialyzed albumin (Fraction V; Sigma Chemical Company, St. Louis, Mo.) and equilibrated against a mixture of O_2 (95%) and CO_2 (5%). In some experiments NaCl (115 mM) was iso-osmotically replaced by sucrose (230 mM), LiCl (115 mM), or choline chloride (115 mM). When complete suppression of sodium was required, sodium bicarbonate (24 mM) was replaced by choline bicarbonate (24 mM; Sigma Chemical Company) while NaCl (115 mM) was replaced by sucrose (230 mM) or LiCl (115 mM). Some media contained no calcium chloride and were enriched with 0.5 or 2.0 mM EGTA (ethylene glycol-bis-(β aminoethyl ether)-N,N′-tetraacetic acid). Results obtained in the presence of the high concentration of EGTA (2.0 mM) were not different from those obtained in the presence of the low concentration of the calcium-chelator so that no further reference to the concentration of EGTA will be made in the text. In all experiments performed in the presence of choline salts, the media also contained atropine sulfate (3.6 μM) in order to prevent cholinergic side effects. The media also contained, as required, glucose, cobalt chloride, and tetrodotoxin (Calbiochem, San Diego, Ca.). All reagents were of analytical grade.

Experimental Procedure

Insulin release and ^{45}Ca efflux from perifused islets. The method used for the measurement of ^{45}Ca efflux and insulin release from perifused islets is described in details elsewhere [18]. Briefly, groups of 100 islets were isolated by the collagenase technique from fed albino rats (body weight: 200–300 g), and incubated for 60 min in the presence of ^{45}Ca (1.12 mM; 0.2 mCi/ml) and glucose (16.7 mM). After incubation, the islets were submitted to five washes with a nonradioactive medium and eventually placed in a perifusion chamber, itself connected to two reservoirs through a three-way valve. The perifusate was delivered at a constant rate (1.0 ml/min), being derived from the first reservoir during the first 44 min and from the second reservoir up to the 70th or 90th min. From the 31st to the 70th or 90th min, the effluent was continuously collected over successive periods of 1 min each in plastic tubes kept at 0 °C. An aliquot of 0.4 ml was then transferred to a counting vial and mixed with 2.5 ml of scintillation fluid (Lumagel, Belgium Lumac S.A., Meise, Belgium). The remainder of the effluent was stored at −20 °C for insulin assay. At the end of the perifusion, the islets and the perifusate remaining in the perifusion chamber were transferred in two separate counting vials and mixed with 10 ml scintillation fluid. In each individual experiment, the efflux of ^{45} Ca (cpm per min) was expressed as a fractional outflow rate (% of islet content per min).

Oxidation of glucose by isolated islets. The method used for the measurement of glucose oxidation has been described in detail elsewhere [27]. In brief, groups of 15 islets each were incubated for 30 or 120 min in small glass tubes themselves placed in scintillation counting vials. The incubation media (0.06 ml) contained [U-^{14}C]glucose (3–10 μCi/ml). After incubation, 0.1 ml HCl (0.2 N) and 0.2 ml hyamine hydroxyde (Packard, Downers Groves, Ill.) were injected through the rubber stopper in the glass tube containing the incubation medium and in the counting vial, respectively. After gentle shaking for 60 min at 20 °C, 10 ml of scintillation fluid (Lipoluma, Packard, Downers Groves, Ill.) were added to the hyamine.

^{45}Ca uptake by isolated islets. The method used for the measurement of ^{45}Ca uptake has been described in detail elsewhere [28]. In brief, groups of 100 islets each were incubated for 90 min in the presence of ^{45}Ca (1.06 mM; 0.1 mCi/ml). After incubation, the islets were submitted to 4 successive washes, transferred in subgroups of 8 islets each in counting vials containing 1 ml distilled water, and examined for their radioactive content by liquid scintillation.

Insulin release. Insulin was radioimmunologically assayed in duplicate with rat insulin as a standard and using a dextran-coated charcoal method for the separation of free and antibody-bound insulin [16].

Radioisotopes. ^{45}Ca was obtained from New England Nuclear (Boston, Mass.), [U-^{14}C] glucose from the Radiochemical Center (Amersham, England) and [^{125}I] insulin from I.R.E. (Fleurus, Belgium).

Statistics. The statistical significance of differences between mean experimental and control data was assessed by use of Student's *t* test.

Results

Lowering Extracellular Sodium Concentration in the Absence of Extracellular Calcium

Partial and iso-osmotic replacement of extracellular sodium by sucrose or lithium so as to reach a 24 mM sodium concentration provoked an immediate and sustained decrease in ^{45}Ca fractional outflow rate (FOR) from islets perifused in the absence of glucose (Fig. 1, upper and middle panels). In both cases, the FOR found 45 min after sodium replacement represented half of the value found at the same time in control experiment ($P < 0.01$; $P < 0.025$). Partial replacement of extracellular sodium by choline also provoked an important reduction in ^{45}Ca FOR (Fig. 1, lower panel). Again the FOR found 45 min after sodium replacement represented half of the value found at the same time in control experiment ($P < 0.01$). The latter decrease in FOR was, however, preceded by an initial and transient increase in ^{45}Ca FOR. A different picture was observed when the same experiments were carried out in the presence of 16.7 mM glucose. Figure 1 shows that lowering the extracellular

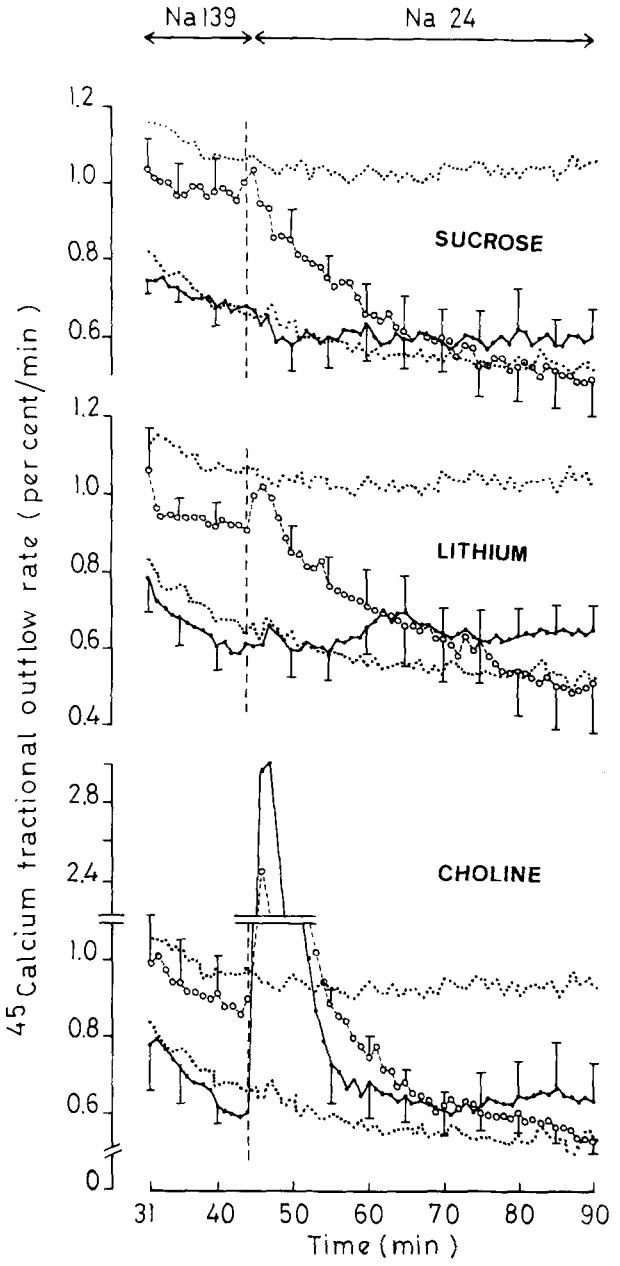

Fig. 1. Effect of partial replacement of sodium chloride by sucrose (230 mM; upper panel), lithium chloride (115 mM; middle panel), and choline chloride (115 mM; lower panel) on ^{45}Ca efflux from rat pancreatic islets perifused with a medium deprived of calcium and enriched with 0.5 mM EGTA. Opencircles (○) refer to experiments made in the absence of glucose. Filled circles (●) refer to experiments made in the presence of 16.7 mM glucose. The stippled lines represent control experiments performed throughout at normal extracellular sodium concentration (139 mM) either in the absence (upper stippled line in each panel) or presence (lower stippled line in each panel) of 16.7 mM glucose. Mean values (±SE of mean) for ^{45}Ca efflux are expressed as a fractional outflow rate (*see* Methods) and refer to 4–6 individual experiments

sodium concentration from 139 to 24 mM failed to decrease ^{45}Ca FOR whether sodium was iso-osmotically replaced by sucrose (upper panel) or lithium (middle panel). Likewise, partial replacement of sodium by choline provoked an initial and transient increase in ^{45}Ca FOR, but the value observed 45 min after sodium replacement was not statistically different from the value found at the same time in control experiment (Fig. 1 lower panel, $P > 0.2$). Comparison of results obtained in the presence and in the absence of glucose revealed that, at normal extracellular sodium concentration, the FOR of ^{45}Ca is significantly lower when the islets were perifused in the presence of 16.7 mM glucose. Thus, the mean value in FOR observed in 9 individual experiments between the 40th and 44th min of perifusion averaged 0.67 ± 0.04 percent per min in the presence of 16.7 mM glucose as distinct from 1.01 ± 0.05 in its absence ($P < 0.001$; *see also* Table 1, lines 6 and 7). Figure 1 further shows that, when sodium was partially replaced by sucrose, lithium, or choline, the ^{45}Ca FOR from islets perifused in the absence of glucose reached a value comparable or even somewhat lower than that observed for islets perifused in the presence of glucose.

Total Removal of Extracellular Sodium in the Absence of Extracellular Calcium

Total replacement of NaCl by sucrose or LiCl and sodium bicarbonate by choline bicarbonate provoked in the absence of glucose a dramatic decrease in ^{45}Ca FOR (Fig. 2, upper panels). This decrease was more marked than that previously seen when sodium was only partially replaced. The FOR found 45 min after total sodium withdrawal was close to 30% of the value found at the same time in control experiments, whether LiCl ($P < 0.001$) or sucrose ($P < 0.005$) in combination with choline were used as the substitute for sodium. In the latter case, the decrease was, however, preceded by a transient increase in ^{45}Ca FOR. Figure 2, lower panels, shows that even total withdrawal of sodium failed to decrease ^{45}Ca FOR below control levels, when the islets were perifused in the presence of 16.7 mM glucose. Again when sucrose in combination with choline were used as the substitute for sodium, an initial and transient increase in ^{45}Ca FOR was noticed.

Lowering Extracellular Sodium Concentration at Normal Extracellular Calcium Concentration

At a normal extracellular calcium concentration (1 mM) the ^{45}Ca FOR was lower in the absence than presence of 16.7 mM glucose (Fig. 3). The mean FOR observed in 8–11 individual experiments between the 40th and 44th min of perifusion averaged $1.20 \pm 0.04\%$ per min in the presence of 16.7 mM glucose

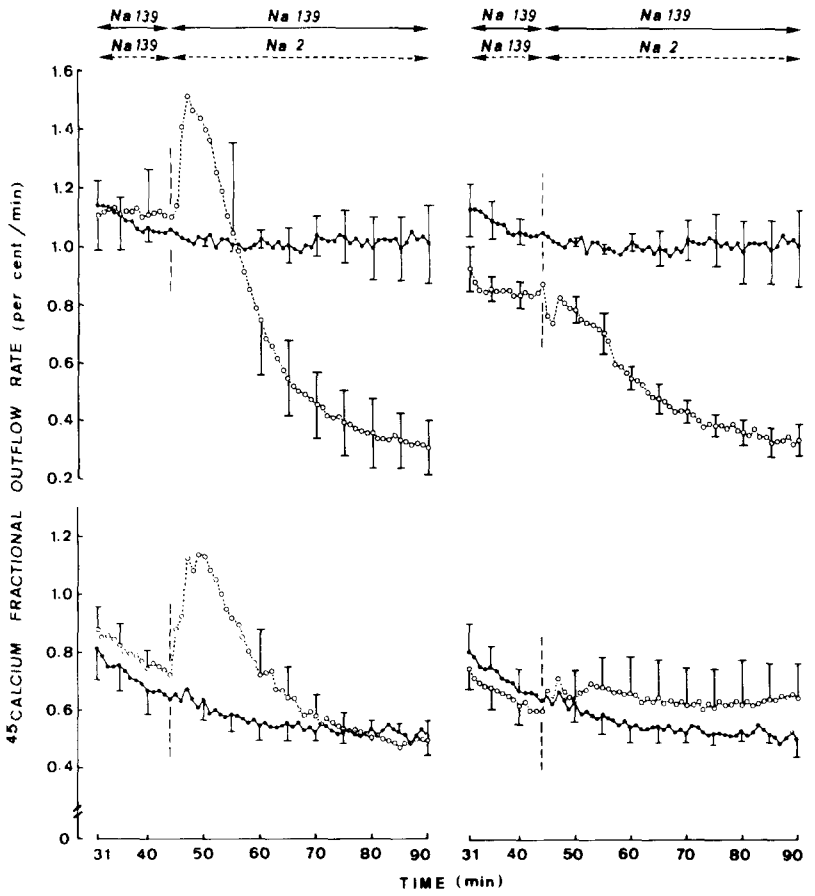

Fig. 2. Effect of total replacement of sodium by the combination of sucrose (230 mM) and choline bicarbonate (24 mM; left panels) or by the combination of lithium chloride (115 mM) and choline bicarbonate (24 mM; right panels) on ^{45}Ca efflux from islets perifused in the absence of calcium (o---o). Control experiments (●——●) were performed in the presence of a normal extracellular sodium concentration throughout. *Upper panels:* the islets were perifused in the absence of glucose; *lower panels:* the islets were perifused throughout in the presence of 16.7 mM glucose. Mean values (±SE of mean) for ^{45}Ca efflux are expressed as in Fig. 1 and refer to 4–5 individual experiments

as distinct from 0.87 ± 0.07 in its absence ($P < 0.001$; *see also* Table 1, lines 1 and 3). Partial replacement of sodium by sucrose provoked an important but transient increase in ^{45}Ca FOR from islets perifused in the absence of glucose (Fig. 3, upper and left panel). Thus 4 min after sodium replacement, the FOR averaged $206.45 \pm 11.83\%$ of the value found at the same time in control experiments. Such an ionic manipulation failed to stimulate insulin release (left and lower panel). In the presence of 16.7 mM glucose, partial replacement of sodium by sucrose also provoked an increase in ^{45}Ca FOR (right and upper panel). The increase was, however, transient and modest, averaging 4 min after sodium replacement $133.11 \pm 9.54\%$ of the value found at the same time in control experiments. This small increase was followed by a slow decrease in FOR below control levels. A significant decrease below control levels was, however, only observed after 23 min of exposure to sodium-depleted media. Insulin release was transiently enhanced by the partial removal of sodium and later reduced, reaching at the 90th min of perifusion a mean value of $40.24 \pm 0.81\%$ of the control value. At a concentration of glucose just below the threshold value for stimulation of insulin release (4.4 mM, Fig. 4), the ^{45}Ca FOR was lower than in the absence of glucose. Indeed, at 4.4 mM glucose, the FOR observed between the 40th and 44th min of perifusion averaged in 10 individual experiments $0.61 \pm 0.05\%$ per min, a value significantly different from the value observed in the absence of glucose ($P < 0.001$; *see* Table 1, lines 1 and 2). In the presence of 4.4 mM glucose throughout, the partial replacement of sodium by sucrose failed to significantly affect ^{45}Ca FOR ($P > 0.5$). Such a ionic manipulation also failed to increase insulin release.

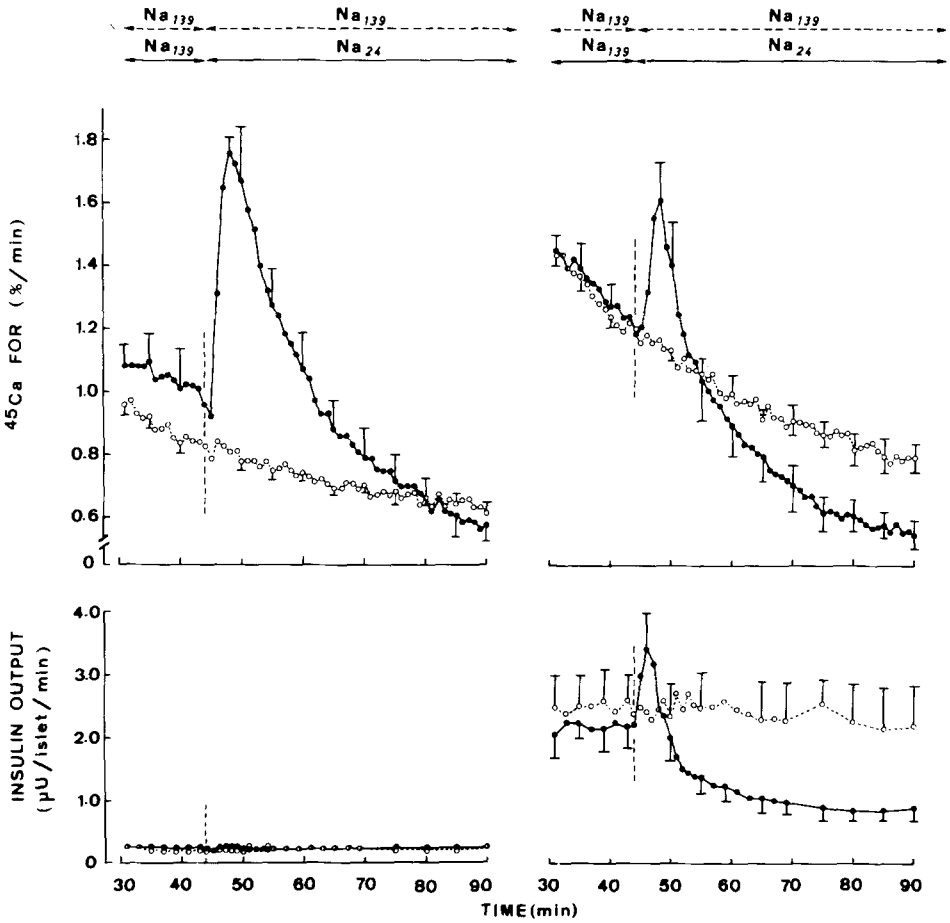

Fig. 3. Effect of partial replacement of sodium by sucrose on ^{45}Ca efflux (upper panel) and insulin release (lower panel) from islets perifused at normal extracellular calcium concentration in either the absence (left panels) or presence of 16.7 mM glucose throughout (●—●; right panels). Control experiments (○---○) were performed in the presence of a normal extracellular sodium concentration throughout, in either the absence (left panels) or presence of 16.7 mM glucose throughout (right panels). Mean values (±SE of mean) for ^{45}Ca efflux are expressed as in Fig. 1 and refer to 5–6 individual experiments. Mean values (±SE of mean) for insulin output are expressed in μU per islet/min and refer to the same 5–6 individual experiments

Table 1. ^{45}Ca FOR from islets perifused at various concentrations of glucose, sodium, calcium, and cobalt[a]

Line	Glucose (mM)	Na^+(mM)	Ca^{2+}(mM)	Co^{2+}(mM)	^{45}Ca FOR	*P*
1	–	139	1	–	0.87±0.04 (19)	
2	4.4	139	1	–	0.61±0.05 (10)	*vs.* line 1 <0.001
3	16.7	139	1	–	1.20±0.04 (11)	*vs.* line 1 <0.001
4	–	139	1	2.5	0.99±0.06 (6)	
5	16.7	139	1	2.5	0.84±0.12 (4)	*vs.* line 4 NS
6	–	139	–	–	0.96±0.04 (27)	
7	16.7	139	–	–	0.65±0.03 (26)	*vs.* line 6 <0.001
8	–	24	1	–	0.57±0.07 (5)	*vs.* line 1 <0.005
9	–	24	–	–	0.61±0.03 (6)	*vs.* line 6 <0.001

[a] Mean values ±SEM for ^{45}Ca FOR were recorded between the 40th and 44th min of perifusion, are expressed in percent per min (*see* Methods), and are shown together with the number of individual observations (*n*) and statistical significance (*P*) of differences between experimental and corresponding control values.

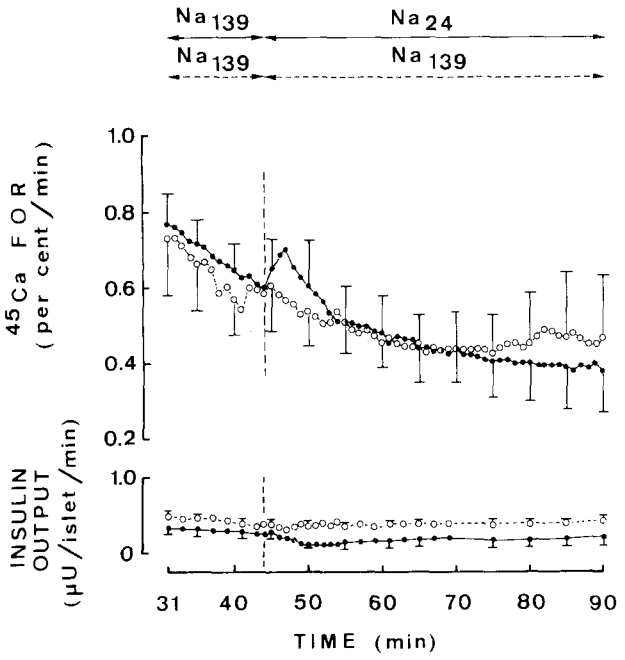

Fig. 4. Effect of partial replacement of sodium by sucrose on ^{45}Ca efflux (upper panel) and insulin release (lower panel) from islets perifused throughout in the presence of 4.4 mM glucose and extracellular calcium (1 mM, ●—●). Control experiments (○---○) were performed in the presence of 4.4 mM glucose and a normal extracellular sodium concentration throughout. Mean values (±SE of mean) for ^{45}Ca efflux and insulin release are expressed as in Fig. 3 and refer to 4–6 individual experiments

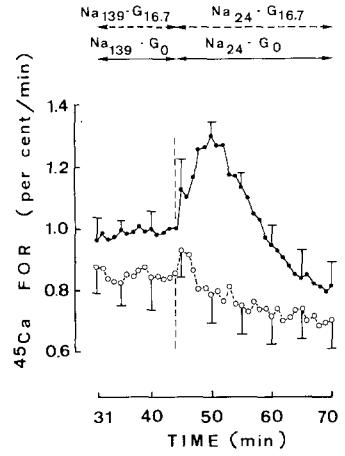

Fig. 5. Effect of partial replacement of sodium by sucrose on ^{45}Ca efflux from islets perifused throughout in the presence of 2.5 mM cobalt and 16.7 mM glucose (○---○) or 2.5 mM cobalt alone (●—●). In both conditions basal perifusate contained calcium (1 mM). Mean values (±SEM) for ^{45}Ca efflux are expressed as in Fig. 1 and refer to 4 individual experiments

Lowering Extracellular Sodium Concentration in the Presence of Cobalt

Cobalt is known to abolish glucose-induced insulin release, presumably by inhibiting calcium entry into the B-cell [15]. In the presence of 2.5 mM cobalt throughout, the ^{45}Ca FOR was higher in the absence than presence of 16.7 mM glucose (Fig. 5; Table 1 lines 4 and 5). Partial replacement of NaCl by sucrose failed to increase the ^{45}Ca FOR from islets perifused in the presence of 16.7 mM glucose, but produced an initial increase followed by a decrease in ^{45}Ca FOR from islets perifused in the absence of glucose.

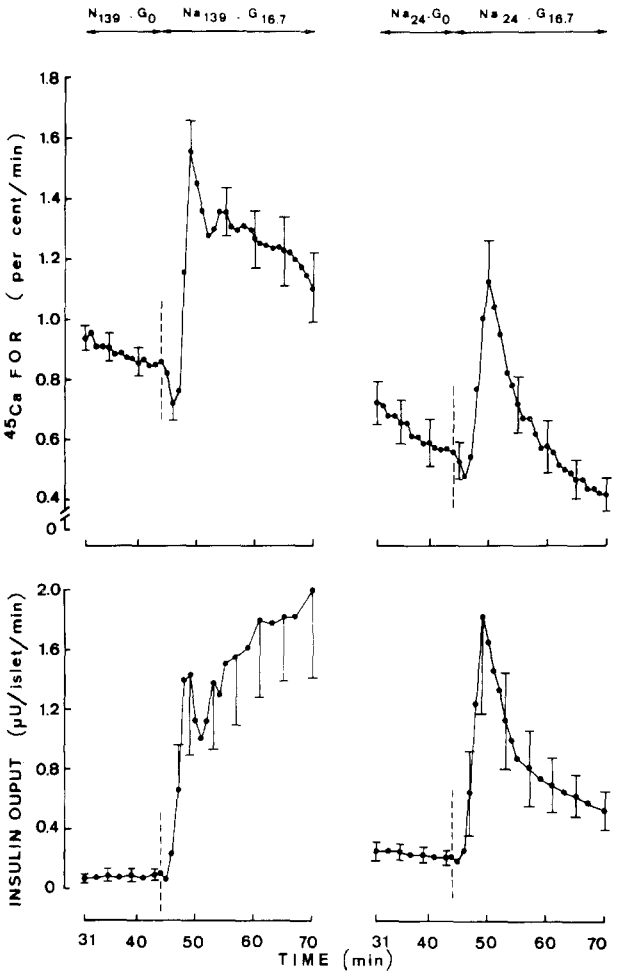

Fig. 6. Effect of 16.7 mM glucose on ^{45}Ca efflux (upper panels) and insulin release (lower panels) from islets perifused throughout at normal (left panels) or low (right panels) extracellular sodium concentration. In both conditions basal perifusate contained calcium (1 mM). Mean values (±SEM) for ^{45}Ca efflux and insulin release are expressed as in Fig. 3 and refer to 4–8 individual experiments

Effect of Glucose on ^{45}Ca Efflux and Insulin Release at Low Extracellular Sodium Concentration

Basal insulin release was somewhat higher at low (Fig. 6, lower and right panel) than at normal extra-

cellular sodium concentration (lower and left panel; $P < 0.05$). 16.7 mM glucose provoked at this low extracellular sodium concentration a monophasic release of insulin. Thus an initial secretory peak comparable to the first phase of insulin release observed in control experiments (Fig. 6, lower and left panel) was elicited, but the usual secondary increase in insulin release could not be detected. The efflux of ^{45}Ca from islets perifused at 24 mM sodium displayed several abnormalities (Fig. 6, right and upper panel). First, the ^{45}Ca FOR observed during the equilibration period was lower than in control experiments carried out at 139 mM sodium (Fig. 6, left and upper panel). The mean value in FOR observed in 5–8 individual experiments between the 40th and 44th min of perifusion averaged $0.57 \pm 0.07\%$ per min at low sodium, as distinct from $0.86 \pm 0.05\%$ per min at normal extracellular sodium concentration ($P < 0.05$; *see also* Table 1, lines 1 and 8). Second, the magnitude of the initial fall in ^{45}Ca FOR was significantly reduced. Thus the mean decrement in ^{45}Ca FOR observed 3 min after glucose introduction averaged $0.22 \pm 0.02\%$ per min at normal extracellular sodium concentration as distinct from 0.11 ± 0.03 at 24 mM extracellular sodium ($P < 0.025$). Third, the secondary rise in ^{45}Ca FOR displayed an altered pattern similar to that of insulin release, being of shorter-than-normal duration. In order to better characterize the effect of a low extracellular sodium concentration upon the magnitude of the initial fall in ^{45}Ca FOR, the experiments were repeated in the absence of extracellular calcium. This procedure is known to suppress the secondary rise without affecting the initial fall in ^{45}Ca efflux [17, 25]. Figure 7 shows that in the absence of glucose (min 31–40) the basal ^{45}Ca FOR was lower at 24 mM (lower panel) than at 139 mM extracellular sodium concentration (upper panel). Thus, the mean value in ^{45}Ca FOR observed in 5–6 experiments between the 40th and 44th min of perifusion averaged 0.79 ± 0.05 at normal as distinct from $0.61 \pm 0.03\%$ per min at low extracellular sodium concentration ($P < 0.025$; *see also* Table 1, lines 6 and 9). At both concentrations of sodium, 16.7 mM glucose reduced ^{45}Ca FOR. The latter effect was, however, strongly impaired and only lasted for about 20 min at low extracellular sodium concentration. Thus, the mean decrement in ^{45}Ca FOR observed in 5–6 individual experiments, 5 min after glucose introduction to the perifusate, averaged $0.33 \pm 0.03\%$ per min at normal as distinct from 0.15 ± 0.01 at low extracellular sodium concentration ($P < 0.001$). Figure 7 further shows that the steady-state level in ^{45}Ca FOR reached in both conditions 10–20 min after introduction of glucose were almost identical ($P > 0.5$).

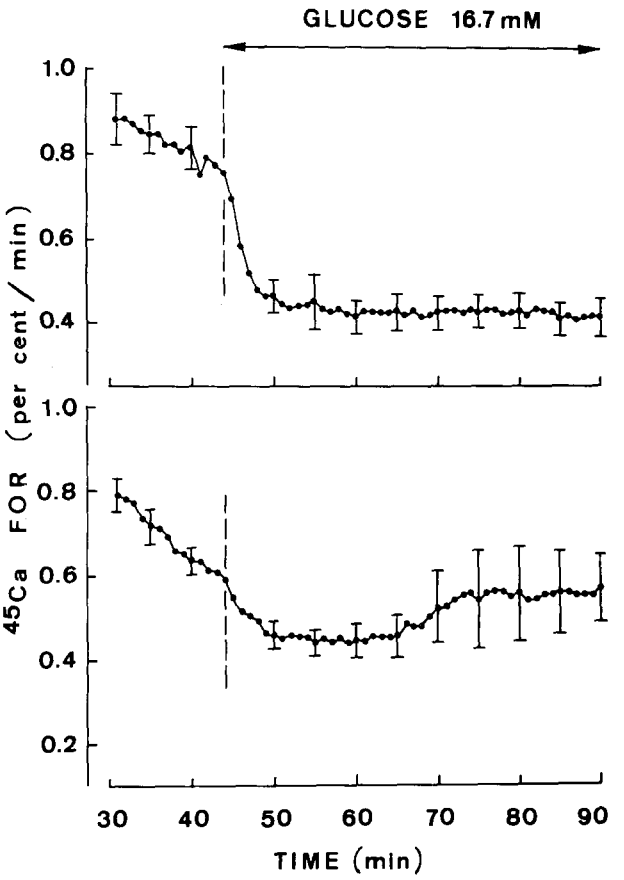

Fig. 7. Effect of 16.7 mM glucose on ^{45}Ca efflux from islets perifused throughout in the absence of extracellular calcium and at normal extracellular sodium concentration (upper panel) or in the absence of extracellular calcium and at low extracellular sodium concentration (lower panel). Mean values (±SEM) for ^{45}Ca efflux are expressed as in Fig. 1 and refer to 5–6 individual experiments

Effect of Tetrodotoxin on Glucose-Induced ^{45}Ca Efflux and Insulin Release

Tetrodotoxin, which selectively blocks sodium channels [4, 20], failed to affect glucose-induced ^{45}Ca efflux (Fig. 8). Thus on addition of 16.7 mM glucose a normal initial inhibition and secondary rise in ^{45}Ca efflux were observed in the presence of 3 μM tetrodoxin. The drug also failed to modify the ^{45}Ca FOR observed in the absence of glucose. Similarly, basal and glucose-stimulated insulin release were comparable whether the islets were perifused in the absence or presence of tetrodotoxin.

Effect of Low Extracellular Sodium and Calcium Concentrations on Glucose Oxidation

At normal extracellular calcium concentration, glucose oxidation was unaffected by a 30-min exposure

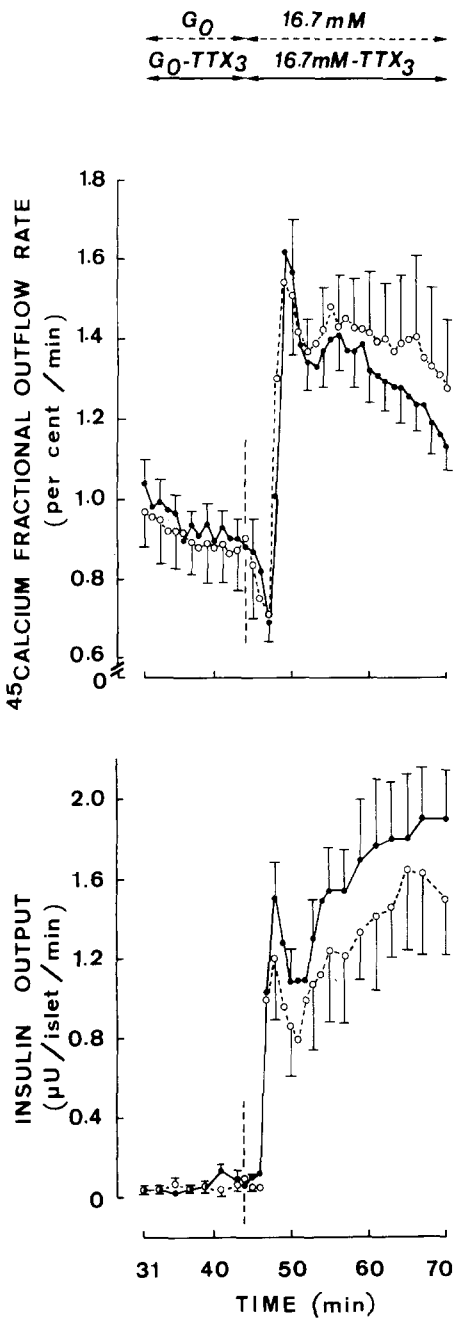

Fig. 8. Effect of 16.7 mM glucose on ^{45}Ca efflux (upper panel) and insulin release (lower panel) from islets perifused in the absence (o---o) or presence (●—●) of tetrodotoxin (3 μM) throughout. Mean values (±SEM) for ^{45}Ca efflux and insulin release are expressed as in Fig. 3 and refer to 4 individual experiments

of the islets to media partially depleted in sodium (Table 2). Prolonged exposure (120 min) to such media, however, provoked a significant fall in glucose oxidation. Over the short incubation period (30 min), exposure of the islets to calcium-depleted media significantly reduced glucose oxidation. The rate of glucose oxidation observed under the latter condition was not, however, further reduced when, in addition, sodium was partially replaced by sucrose.

Effect of Glucose, Cobalt and a Low Extracellular Sodium Concentration on ^{45}Ca Net Uptake by Isolated Islets

At normal extracellular sodium and calcium concentrations, glucose caused a dose-related stimulation of ^{45}Ca net uptake. When the extracellular sodium concentration was decreased, ^{45}Ca net uptake was dramatically increased in the absence of glucose. In the presence of glucose, the increase in ^{45}Ca net uptake attributable to a decrease in extracellular sodium concentration failed to reach statistical significance. Cobalt markedly decreased the net uptake of ^{45}Ca observed at low sodium concentration, whether in the absence or presence of glucose. At the low sodium concentration and in the absence of cobalt, the net uptake of ^{45}Ca was much lower in the presence of 5.6 mM glucose than either in the absence of the sugar or in the presence of 16.7 mM glucose.

Discussion

In the absence of extracellular calcium, i.e., in a condition known to prevent calcium-calcium exchange [17], the ^{45}Ca FOR from islets perifused in the absence of glucose averaged $0.95 \pm 0.04\%$ per min, 40 to 44 min after the onset of the perifusion. 16.7 mM glucose reduced this ^{45}Ca FOR by about 30%, confirming previous observations that in the absence of extracellular calcium glucose exerts a sustained inhibitory effect on ^{45}Ca efflux from preloaded islets [13, 18, 22, 25]. Partial or total replacement of sodium by sucrose or lithium provoked a graded reduction in ^{45}Ca FOR from islets perifused in the absence of glucose. Such a phenomenon failed to occur in the presence of 16.7 mM glucose. Moreover, when extracellular sodium was partially removed, the ^{45}Ca FOR from islets perifused in the absence of glucose reached values similar to that from islets perifused in the presence of glucose (Fig. 1). These findings indicate the existence in islet cells of a carrier-mediated exchange of sodium for calcium. This cationic exchange may be responsible for at least 70% of the efflux of ^{45}Ca since in the complete absence of extracellular sodium the FOR of ^{45}Ca only represented 30% of the value observed at normal extracellular sodium concentration (Fig. 2). Glucose inhibits such a carrier-mediated exchange of sodium for calcium, the effect of the sugar in inhibiting this process being almost as marked as that seen in response to a lowering of

Table 2. Effect of partial replacement of sodium by sucrose on glucose oxidation in the absence or presence of extracellular calcium[a]

Line	Glucose (mM)	Na^+ (mM)	Ca^{2+} (mM)	EGTA (mM)	Time of incubation (min)	Glucose oxidation		P	
						fmol/min per islet	% of control value		
1	16.7	139	1	–	30	593±67 (7)			
2	16.7	24	1	–	30		107.3±9.6 (7)	*vs.* line 1	NS
3	16.7	139	–	1	30		74.2±4.3 (17)	*vs.* line 1	<0.005
4	16.7	24	–	1	30		78.5±7.1 (17)	*vs.* line 3	NS
5	16.7	139	1	–	120	720±30 (15)			
6	16.7	24	1	–	120		69.0±6.0 (13)	*vs.* line 5	<0.001

[a] Mean values (±SEM) are shown together with the number of individual observations (*n*) and statistical significance (*P*) of differences between paired values.

the extracellular sodium concentration from 139 to 24 mM. The finding that this ionophoretic process is inhibited by reducing the extracellular sodium concentration to 24 mM is compatible with a high sodium concentration inside the islet cells (approximately 50 mM; *see* refs. 21, 32).

Similar results were observed when choline was used as the substitute for extracellular sodium. In the latter case, however, an initial and transient increase in ^{45}Ca efflux was usually observed. The absence of such an initial increase in ^{45}Ca efflux when sodium was partially replaced by sucrose or lithium argues for a specific effect of choline on ^{45}Ca handling, as already postulated elsewhere [31]. The enhancing effect of choline on ^{45}Ca efflux was related to the concentration of choline (24–115 mM; *see* Figs. 1 and 2) and was antagonized by lithium (Fig. 2), suggesting a possible dependency of this effect on the rate of choline entry in islet cells. Incidentally, choline also differs from lithium or other substitutes for extracellular sodium in that replacement of sodium by choline increases glucose-induced insulin release, whereas the opposite picture is observed when sodium is replaced by Tris, lithium, or potassium [26]. In order to avoid specific effects of the sodium substitute, sucrose was exclusively used to replace extracellular sodium in further experiments.

At normal extracellular calcium concentration (1.0 mM), the ^{45}Ca FOR from islets was increased by about 40% as the concentration of glucose was raised from zero to 16.7 mM (Fig. 6 and Table 1). This confirms that, at normal extracellular calcium concentration, the overall effect of glucose consists in a sustained enhancement of ^{45}Ca efflux [19]. This overall enhancement is thought to represent the balance between an increase in ^{45}Ca efflux due to stimulation of a ^{40}Ca–^{45}Ca exchange process [17], and a concomitant decrease attributable to inhibition of the process of sodium-calcium exchange. The latter process could correspond to a process of Na-Ca counter-transport resembling that described in nerve tissue [1, 5]. Under physiological conditions, i.e., at normal sodium and calcium concentrations, this process exchanges effluent calcium for influent sodium. In these tissues, when the extracellular sodium concentration is sufficiently reduced, the same carrier system may transport calcium into the cell [2]. This also appears to be the case in the islet cells. Indeed, when NaCl was partially replaced by sucrose, an increase in ^{45}Ca FOR was first observed. The view that this initial increase is indeed attributable to facilitated influx of ^{40}Ca into the islet cells is supported by the fact that the phenomenon failed to occur when NaCl was replaced by sucrose in the absence of extracellular calcium (*see* Fig. 1). The facilitation of calcium influx may also account, in part at least, for a trend towards an increased net uptake of ^{45}Ca in the islets incubated at low extracellular sodium concentration (Table 3).

Table 3. Effect of partial replacement of Na^+ by sucrose and of cobalt on ^{45}Ca uptake at three glucose concentrations[a]

Glucose	139 mM Na^+	24 mM Na^+	24 mM Na^+ +2.5 mM Co^{2+}
Nil	1.62±0.15 (12)	5.86±0.30 (36)	2.97±0.19 (12)
5.6 mM	3.42±0.13 (34)	3.81±0.24 (24)	–
16.7 mM	6.42±0.23 (33)	6.74±0.25 (36)	3.82±0.32 (12)

[a] Mean values (±SEM) are expressed as pmol/90 min per islet.

At a 4.4 mM glucose concentration, which is close to the threshold value for stimulation of insulin release, glucose exerts a marked inhibitory effect on ^{45}Ca efflux but fails to provoke a secondary increase in effluent radioactivity [19]. The present observation that the ^{45}Ca FOR was significantly lower in the presence of 4.4 mM glucose than in the absence of glucose confirms that, at this critical concentration, glucose exerts a sustained inhibitory effect on ^{45}Ca efflux. In the presence of 4.4 mM glucose the replacement of NaCl by sucrose failed to provoke any in-

crease in ^{45}Ca FOR. This finding is compatible with the view that glucose inhibits the activity of the sodium-calcium counter transportprocess and that the dose-action relationship for such an inhibitory effect is superimposable to the dose-action relationship characterizing the inhibitory component of the glucose effect upon ^{45}Ca efflux [19].

If the activity of the sodium-calcium countertransport process is sufficiently reduced at 4.4 mM glucose as to prevent any increase in ^{45}Ca FOR on partial removal of extracellular sodium, it may appear surprising that such an increase occurred at 16.7 mM glucose (Fig. 3, right panel). However, at high glucose concentration, an increase in ^{40}Ca influx and subsequent ^{45}Ca efflux can take place through calcium channels which are gated by glucose concentrations above 5.6 mM. This view, which implies that extracellular sodium also affects the rate of calcium transport by these gated calcium channels, is supported by the observation that cobalt, which is claimed to selectively block calcium channels [3], completely suppressed the effect of a low extracellular sodium concentration on ^{45}Ca FOR when the islets were perifused in the presence of 16.7 mM glucose.

In the absence of glucose, cobalt failed to suppress the increase in ^{45}Ca FOR due to substitution of NaCl by sucrose. It should be noted, however, that, in the glucose-deprived islets, cobalt significantly reduced the magnitude of the rise in ^{45}Ca FOR attributable to sodium replacement, suggesting that cobalt may also compete with calcium at the level of the sodium-calcium countertransport process as already reported by Baker in nerve tissue [1].

The effect of cobalt upon ^{45}Ca FOR is also consonant with the postulated role of glucose to increase calcium entry into the islet cells. Thus, in the absence of glucose, cobalt failed to markedly affect ^{45} Ca FOR, as measured between the 40th and 44th min of perifusion. In contrast, in the presence of glucose, cobalt dramatically reduced ^{45}Ca FOR, which now became even lower, although not significantly so, than the mean value recorded in the absence of glucose but presence of cobalt (Table 1, lines 4 and 5).

In all experiments performed at normal extracellular calcium concentration, there was a close similarity between the capacity of a given experimental situation (e.g., decrease in extracellular sodium concentration; presence of cobalt; changes in glucose concentration) to stimulate ^{40}Ca–^{45}Ca exchange in the perifused islets and to enhance ^{45}Ca net uptake by incubated islets, respectively. In this respect, a striking feature consisted in the much lower net uptake of ^{45}Ca found at 5.6 mM glucose as distinct from either zero or 16.7 mM glucose in the islets exposed to a low extracellular sodium concentration. This situation is reminiscent of that characterizing the stimulation of ^{45}Ca FOR in response to a decrease in extracellular sodium concentrations. Indeed, and as already underlined, such an increase in ^{45}Ca FOR was fairly obvious in the absence of glucose or at 16.7 mM glucose (Fig. 6) but virtually absent at an intermediate glucose concentration (Fig. 7). Likewise, cobalt decreased but failed to abolish the enhancement of both ^{45}Ca FOR and ^{45}Ca net uptake evoked by a fall in extracellular sodium concentration in glucose-deprived islets. Last, in the islets exposed to 16.7 mM glucose, cobalt severely reduced both ^{45}Ca FOR and ^{45}Ca net uptake and abolished the increase in ^{45}Ca FOR evoked by a decrease in sodium concentration.

The participation of the sodium-calcium countertransport process in the cationic response of the islets to glucose was further documented by characterizing the effect of a rise in glucose concentration upon ^{45}Ca efflux at different concentration of extracellular sodium. The inhibitory effect of glucose on ^{45}Ca FOR from pancreatic islets was reduced by about 50% at low extracellular sodium concentration whether in the absence or presence of extracellular calcium. This confirms the hypothesis that glucose exerts its inhibitory effect on ^{45}Ca efflux by reducing the activity of the sodium-calcium countertransport process. In the presence of extracellular calcium, glucose-stimulated a secondary rise in ^{45}Ca FOR. This secondary rise was of lesser magnitude and shorter duration at low than at normal sodium concentration. Similarly, the secretory response to glucose was shortened at low sodium concentration. Thus, whereas a normal if not enhanced initial secretory peak was observed, the usual secondary increase in insulin release was virtually absent. Furthermore, the inhibitory effect of glucose on ^{45}Ca FOR observed in the absence of calcium was not only reduced at low sodium concentration, but it was also of short duration. These late anomalies are compatible with a delayed alteration of electrical [9], biosynthetic [31], and metabolic events [14]. The rate of glucose oxidation was indeed decreased when the islets were exposed during 120 min to media partially depleted in sodium, but unaffected after only 30 min incubation at low sodium concentration. This delayed alteration of metabolic events could also explain why partial replacement of sodium by sucrose resulted in a delayed decrease in insulin release and ^{45}Ca FOR from islets perifused in the presence of 16.7 mM glucose and 1 mM calcium (Fig. 3). The absence of alteration in the rate of glucose oxidation during short-term incubation (30 min) in media partially depleted in sodium suggests that none of the immediate effects of such an ionic manipulation on calcium fluxes and insulin release is due to alteration of glucose metabolism.

In a recent report it was speculated that glucose stimulated sodium entry into the B-cell through veratridine-sensitive sodium channels and that the resulting increase in intracellular sodium concentration might inhibit ^{45}Ca efflux from pancreatic islets by competition of sodium with calcium for the common carrier system [10]. In nerve tissue intracellular sodium when reaching a sufficiently high concentration may indeed reduce calcium efflux [6]. In nerve tissue also, tetrodotoxin blocks the major sodium channel [4, 20], but there is no evidence that it interferes directly with the carrier system which is responsible for sodium-calcium countertransport and which only accounts for a minor fraction of the total rate of sodium influx [6]. The same situation apparently prevails in the islets. On one hand, tetrodotoxin blocks the veratridine-sensitive channel and by doing so blocks veratridine-induced insulin release [10]. On the other hand, tetrodotoxin failed to modify the normal biphasic pattern of ^{45}Ca efflux induced by 16.7 mM glucose. Tetrodotoxin also fails to affect the ability of glucose to depolarize the B-cell plasma membrane [29] and, except in one study [10], did not modify glucose-stimulated insulin release (Fig. 8; and [30]). These converging findings do not suggest that changes in the rate of sodium entry play a major role in the mechanism by which glucose initiates insulin release, and do not support the idea that glucose inhibits sodium-calcium countertransport by increasing the intracellular sodium concentration. Incidentally, it was recently reported that, under steady-state conditions, glucose, although indeed increasing sodium inflow, moderately reduces the concentration of sodium inside the islet cells [21].

It could be argued that most of the effects observed in response to a decrease in the extracellular sodium concentration are the result of a fall in the intracellular concentration of sodium in the islet cells and of a subsequent decrease in the exchange of sodium for calcium at the level of intracellular organelles. The existence of such a process of sodium-calcium exchange, initially described by Carafoli [8] in heart mitochondria has also been postulated in the B-cell [10]. The latter process would, however, hardly account for the increase in calcium influx and, hence ^{45}Ca efflux observed at normal extracellular calcium concentration when the islets were exposed to a low concentration of extracellular sodium.

It is unlikely that, in the absence of glucose, the changes in calcium efflux evoked by a decrease in extracellular sodium concentration are attributable to altered membrane potential. Thus, according to Dean and Matthews [9], removal of extracellular sodium does not produce a significant fall in membrane potential in the presence of a low glucose concentration. In the presence of 11.1 mM glucose, however, removal of extracellular sodium suppresses the periodic repolarization phase normally seen in between burst of spikes and, hence, causes a continuous spike activity [9, 29]. This phenomenon, attributed by Meissner and Preissler [29] to an inhibition of the sodium-potassium pump as a consequence of a decreased intracellular sodium activity, may contribute to the increase in calcium inflow (and hence ^{40}Ca–^{45}Ca exchange) through voltage-sensitive calcium channels here observed at high glucose concentration (Fig. 3, right panel).

In conclusion, the present work provides evidence for the existence in the pancreatic B-cell of a process of sodium-calcium countertransport. This process may be responsible for a major fraction of the efflux of ^{45}Ca. Inhibition of such a process appears to be the mechanism by which glucose reduces the efflux of Ca from pancreatic islets. The latter phenomenon is apparently not mediated by an increase in the intracellular sodium concentration. It is conceivable that the opening of the channels mediating calcium entry into the islet cells and the inhibition of the sodium-calcium countertransport both participate in the glucose-induced intracellular accumulation of calcium and subsequent release of insulin

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