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GLUCOSE INHIBITS ^{45}Ca EFFLUX FROM PANCREATIC β-CELLS ALSO IN THE ABSENCE OF Na^+-Ca^{2+} COUNTERTRANSPORT

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During perifusion with medium deprived of Ca^{2+}, addition of glucose or omission of Na^+ resulted in prompt and quantitatively similar inhibitions of ^{45}Ca efflux from β-cell rich pancreatic islets microdissected from *ob / ob* mice. Glucose had no additional inhibitory effect when Na^+ was isoosmotically replaced by sucrose or choline$^+$. When K^+ was used as a substitute for Na^+, the inhibitory effect of Na^+ removal on ^{45}Ca efflux became additive to that of glucose. The observation that glucose can be equally effective in inhibiting ^{45}Ca efflux in the presence or absence of Na^+ is difficult to reconcile with the postulate that the Na^+-Ca^{2+} countertransport mechanism is a primary site of action for glucose.

Introduction

Glucose is the major physiological stimulant for insulin release. As in other secretory processes the discharge of the hormone is supposed to be activated by an increase of the Ca^{2+} activity in the cytosol [1–3]. The ionic events involved in the coupling of recognition of glucose to insulin discharge have been the subject of extensive investigation. By monitoring the ^{45}Ca washout from preloaded pancreatic islets it has been possible to demonstrate two distinct actions of glucose in terms of a rapid inhibition masked by a secondary rise of the efflux of radioactivity [1,3,4–6].

Whereas the stimulatory phase of ^{45}Ca efflux probably represents a process of Ca^{2+}-Ca^{2+} exchange in which entering non-radioactive Ca^{2+} displaces ^{45}Ca from intracellular binding sites [5,6], divergent opinions have been expressed about the mechanism involved in inhibition of the efflux. The glucose inhibition of ^{45}Ca efflux has been attributed either to suppression of Na^+-Ca^{2+} countertransport across the plasma membrane [3,7] or to increased sequestration of the ion in organelles [4,8]. It is obvious that the two alternatives are fundamentally different in having opposite effects on the cytosolic Ca^{2+} activity.

The major arguments for a glucose inhibition of the Na^+-Ca^{2+} countertransport mechanism are the observations when using Ca^{2+}-deficient media that addition of glucose has little effect on ^{45}Ca efflux if the concentration of extracellular Na^+ is reduced and that omission of Na^+ fails to inhibit the radioactive outflow from islets exposed to a high concentration of glucose [7]. With the present demonstration that glucose is equally effective in inhibiting the ^{45}Ca efflux in the presence or absence of Na^+ when the cytoplasmic K^+ activity is maintained, it is unlikely that the Na^+-Ca^{2+} countertransport mechanism is the site of action for glucose.

Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Materials and Methods

Adult *ob/ob*-mice were taken from a non-inbred colony [9] and starved overnight. The animals were killed by decapitation and pancreatic islets were isolated by microdissection. The basal medium used for the isolation and the subsequent measurements of ^{45}Ca efflux was a Hepes buffer with Cl^- as the sole anion [10]. The pH of this buffer was adjusted to 7.4 by addition of either approx. 12 mM NaOH, or KOH (when preparing a completely Na^+-free medium).

The kinetics of ^{45}Ca efflux were studied using previously described procedures [11]. The islets were incubated with 1.28 mM ^{45}Ca (391 Ci/mol) for 90 min. After two serial 5-min washes, batches of 8–10 islets were transferred to a 10 μl chamber and perifused at a constant rate of about 40 μl/min with a non-radioactive medium deficient in Ca^{2+} and supplemented with 0.5 mM EGTA and 1 mg/ml albumin (final Ca^{2+} activity < 0.01 μM). When the effect of a reduced Na^+ concentration was studied, osmotic and/or ionic compensation was achieved by isoosmotic replacement of NaCl with sucrose, choline chloride or KCl. Details about such modifications and the concentrations of glucose are given in the legends to the figures and Table I. In each experiment, two or three chambers were loaded with islets from the same animal and run in parallel. The perifusate was collected over successive periods of 2 or 5 min and samples analysed for radioactivity by liquid scintillation counting. After perifusion, the islets were removed from the chambers, freeze-dried overnight and weighed on a quartz-fibre balance. Statistical significance of effects was assessed from the differences between paired test and control data using the two tailed Student's distribution.

Results

The effects of lowering the Na^+ concentration to 12 mM on the efflux of ^{45}Ca into a Ca^{2+}-deficient medium is shown in Fig. 1. Isoosmotic replacement of Na^+ with K^+ or choline$^+$ was equally effective in reducing ^{45}Ca efflux. The addition of glucose resulted in a further inhibition of the radioactive efflux provided that the reduced Na^+ was compensated osmotically by replacement

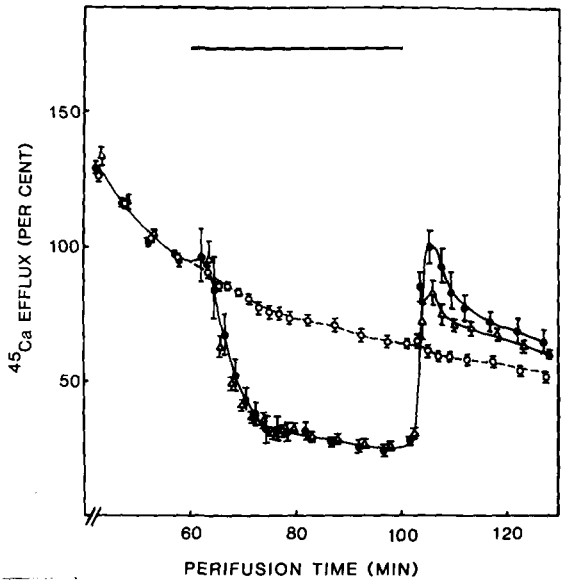

Fig. 1. Effects of substituting Na^+ with K^+ or choline$^+$ on ^{45}Ca efflux in Ca^{2+}-deficient media. The islets were loaded with 1.28 mM ^{45}Ca (391 Ci/mol) for 90 min in the presence of 20 mM glucose and then perifused with a Ca^{2+}-deficient medium supplemented with 0.5 mM EGTA. During the period indicated by the horizontal bar the Na^+ concentration was either maintained (○) or reduced to 12 mM by isoosmotic replacement with K^+ (●) or choline$^+$ (△). The data are given as a percentage of the average ^{45}Ca efflux in the individual experiments during the 10 min preceding the introduction of glucose. Mean vaues ± S.E. for five experiments.

with K^+ but not with choline$^+$ or sucrose (Fig. 2). Inhibition of ^{45}Ca efflux was also observed when most of the Na^+ was substituted with K^+ in a medium containing glucose (Fig. 3). The ability of glucose to inhibit ^{45}Ca efflux was maintained after complete replacement of Na^+ with K^+. As shown in Fig. 4, glucose was as effective in reducing ^{45}Ca efflux in a Na^+-depleted perifusion medium as when the original Na^+ concentration (137 mM) was used.

In Fig. 5 a comparison is made between the inhibition of ^{45}Ca efflux obtained in a Ca^{2+}-deficient medium with glucose exposure and that observed when substituting all Na^+ with K^+. Both alternatives were almost as effective in suppressing the radioactive efflux. The combination of the two procedures resulted in greater inhibition than obtained with each alone. It was consequently possible to demonstrate a significant glucose potentiation of the effect of Na^+ removal already 5 min

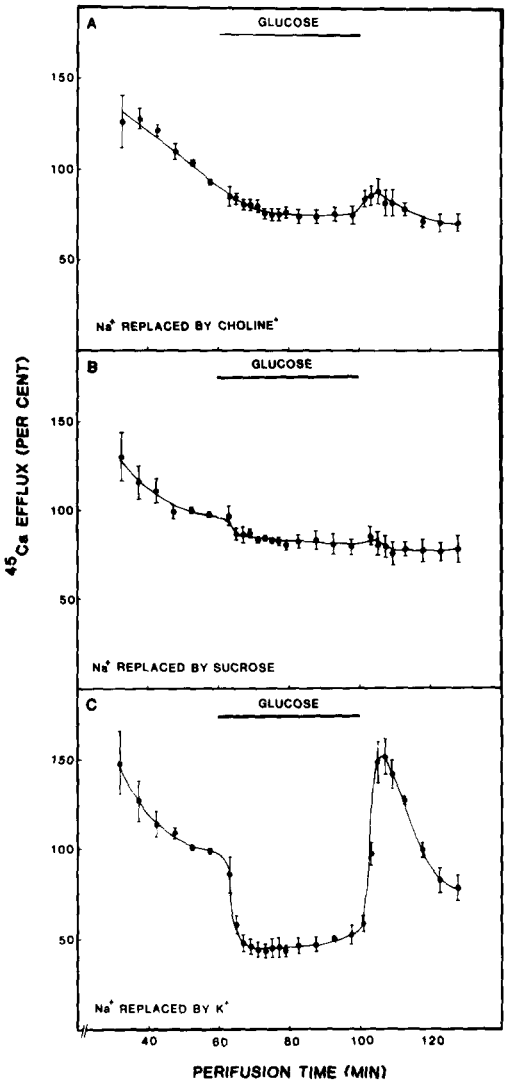

Fig. 2. Effect of glucose on ^{45}Ca efflux in Ca^{2+}-deficient media with reduced Na^+. The islets were loaded with 1.28 mM ^{45}Ca (391 Ci/mol) for 90 min in the presence of 20 mM glucose and then perifused with a Ca^{2+}-deficient medium supplemented with 0.5 mM EGTA and containing only 12 mM Na^+. The reduction of the Na^+ concentration from the normal 137 mM was done by isoosmotic replacement of NaCl with choline chloride (panel A), sucrose (panel B) or KCl (panel C). Glucose (20 mM) was added to the perifusion media during the period indicated by the horizontal bars. The data are given as a percentage of the average ^{45}Ca efflux in the individual experiments during the 10 min preceding the introduction of glucose. Mean values ± S.E. for four experiments.

after changing the composition of the perifusion medium ($P < 0.001$). Table I refers to similarly designed experiments indicating the glucose effect

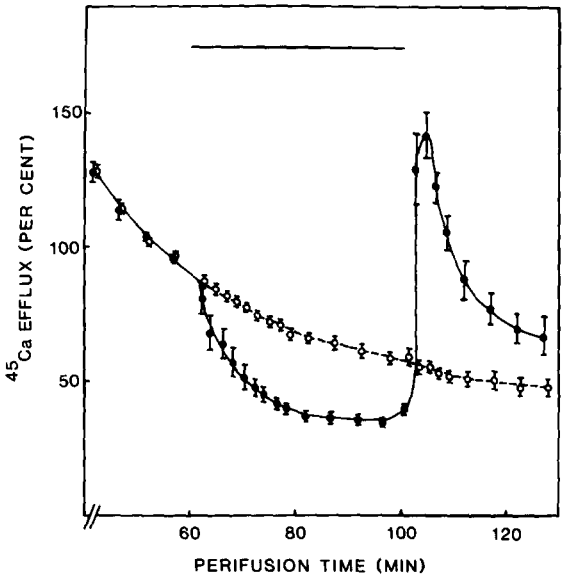

Fig. 3. Effects of substitution of Na^+ with K^+ on ^{45}Ca efflux in Ca^{2+}-deficient media containing glucose. The islets were loaded with 1.28 mM ^{45}Ca (391 Ci/mol) for 90 min in the presence of 20 mM glucose and then perifused with a Ca^{2+}-deficient medium supplemented with 0.5 mM EGTA and 20 mM glucose. During the period indicated by the horizontal bar the Na^+ concentration was either maintained (○) or reduced to 12 mM by isoosmotic replacement with K^+ (●). The data are given as a percentage of the average ^{45}Ca efflux in the individual experiments during the 10 min preceding the introduction of glucose. Mean values ± S.E. for seven experiments.

on ^{45}Ca efflux 25 min after simultaneous reduction of Na^+ to 12 mM. Again the inhibitory effect of glucose was critically dependent on a high K^+ concentration in the Na^+-deficient medium; no suppression was observed after substituting Na^+ with choline$^+$ or sucrose.

Discussion

Glucose inhibition of ^{45}Ca efflux is a rapidly established and sustained phenomenon which is preferably studied in the absence of extracellular Ca^{2+} [5,6,11]. The dose-effect relationship for glucose is hyperbolic with a K_m of 4.2 mM [5], indicating that this function is vastly different from the sigmoidal relationship for glucose-stimulated ^{45}Ca efflux and insulin release with K_m of about 9 mM. Since the inhibition of ^{45}Ca efflux is observed already at concentrations of glucose which do not stimulate insulin secretion [5,6], it

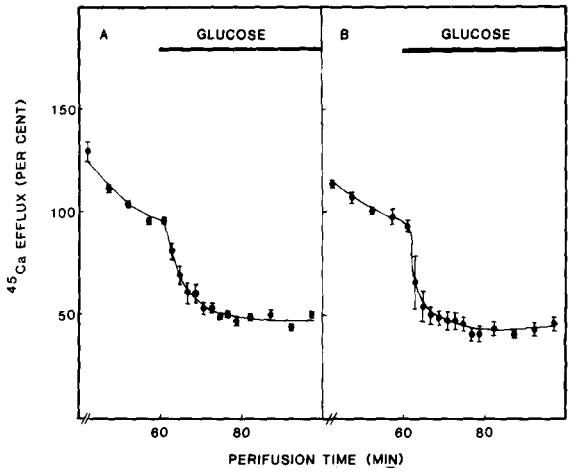

Fig. 4. Effect of glucose on ^{45}Ca efflux in Ca^{2+}-deficient media with Na^+ or Na^+ replaced by K^+. The islets were loaded with 1.28 mm ^{45}Ca (391 Ci/mol) for 90 min in the presence of 20 mM glucose and then perifused with a Ca^{2+}-deficient medium supplemented with 0.5 mM EGTA and either containing normal concentration of Na^+ (137 mM; panel A) or lacking the ion after isoosmotic replacement with K^+ (panel B). Glucose (20 mM) was added to the perifusion media during the period indicated by the horizontal bars. The data are given as a percentage of the average ^{45}Ca efflux in the individual experiments during the 10 min preceding the introduction of glucose. Mean values ± S.E. for five experiments.

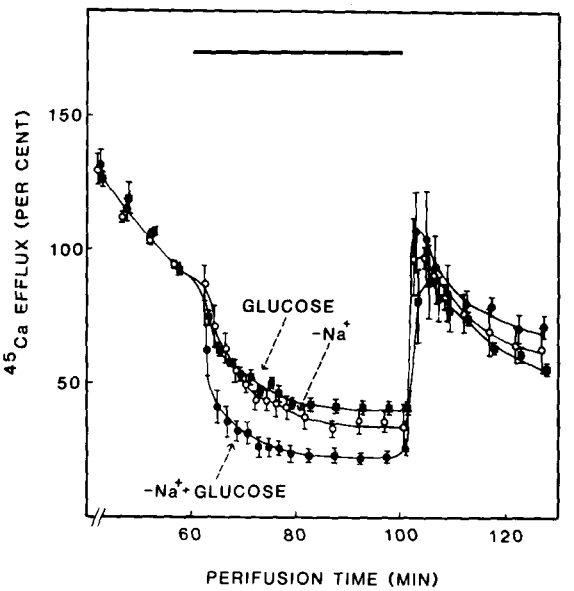

Fig. 5. Effects of glucose and substitution of Na^+ for K^+ on ^{45}Ca efflux in Ca^{2+}-deficient media. The islets were loaded with 1.28 mM ^{45}Ca (391 Ci/mol) for 90 min in the presence of 20 mM glucose and then perifused with a Ca^{2+}-deficient medium supplemented with 0.5 mM EGTA. During the period indicated by the horizontal black bar the media were modified by excluding Na^+ (isoosmotic replacement with K^+) and/or adding 20 mM glucose. The data are given as a percentage of the average ^{45}Ca efflux in the individual experiments during the 10 min preceding the introduction of glucose. Mean values ± S.E. for five experiments.

may be related to the initial depolarization [12,13] preceding the opening of the voltage-dependent Ca^{2+} channels.

When comparing the inhibitory effects of glucose on ^{45}Ca efflux with that obtained after Na^+ reduction it is important to make osmotic and/or ionic compensations for the omitted Na^+. In previous experiments [7,14] such compensation was made by the addition of sucrose, choline$^+$ or Li^+.

TABLE I

EFFECTS OF GLUCOSE ON THE SUPPRESSION OF ^{45}Ca EFFLUX OBTAINED BY SIMULTANEOUS REPLACEMENT OF MOST OF THE Na^+ WITH SUCROSE, CHOLINE$^+$ OR K^+

The islets were loaded with 1.28 mM ^{45}Ca (391 Ci/mol) for 90 min in the presence of 20 mM glucose and then perifused with glucose-free and Ca^{2+}-deficient medium supplemented with 0.5 mM EGTA. After 60 min of perifusion the medium concentration of Na^+ was reduced to 12 mM by isoosmotic replacement of NaCl with sucrose, choline chloride or KCl and 20 mM glucose was simultaneously added to some of the perifusates. The figures indicate the rate of ^{45}Ca efflux 25 min after the reduction of Na^+ and are expressed as percentage of the average ^{45}Ca efflux in the individual experiments during the 10 min preceding the change of medium. Mean values ± S.E. * $P < 0.05$; ** $P < 0.001$.

Substitute for Na^+	n	Percentage value after 25 min		
		Without glucose	With glucose	Effect of glucose
Na^+ (control)	7	59.2 ± 1.9	39.3 ± 1.5	−19.9 ± 2.9 **
Sucrose	4	30.3 ± 3.1	27.6 ± 3.2	−2.7 ± 5.0
Choline$^+$	5	30.3 ± 3.8	23.3 ± 4.0	−6.9 ± 4.5
K^+	4	33.7 ± 3.6	24.6 ± 4.8	−9.1 ± 2.5 *

Apart from the immediate reduction of Ca^{2+} efflux, a decrease of extracellular Na^+ will result in intracellular Na^+ depletion with subsequent inhibition of the Na^+/K^+ pump [15,16]. A decrease of cytosolic K^+ is therefore an expected consequence of reduced extracellular Na^+. A maintained K^+ activity has previously been shown to be important for glucose metabolism of the β-cells [17]. Furthermore, the ion stimulates uptake of Ca^{2+} both into chromaffin granules [18] and subcellular particles of β-cells [19]. To test the possibility that a decrease of cytosolic K^+ may have led to incorrect conclusions about the role of glucose in Na^+-Ca^{2+} countertransport we have now also used K^+ when compensating for the reduced Na^+ concentration.

In accordance with previous observations [7,14], the addition of glucose or reduction of Na^+ were found to have similar actions in reducing ^{45}Ca efflux into Ca^{2+}-depleted medium. It was also possible to confirm that glucose lacked significant inhibitory effects when added to media where Na^+ had been replaced by choline$^+$ or sucrose. However, when K^+ was used to compensate for the Na^+ reduction, glucose inhibited ^{45}Ca efflux markedly. Under the latter condition the inhibitory effects of Na^+ omission and glucose addition indeed appeared to be additive. The outward transport of ^{45}Ca was inhibited to the same extent when K^+ and choline$^+$ were used as substitutes for Na^+. It seems consequently unlikely that K^+ could substitute significantly for Na^+ in Na^+-Ca^{2+} countertransport. The K^+ affinity of this exchange is low [20] and estimates of intracellular K^+ [1,21] indicate that the required inward K^+ gradient is absent also when all extracellular Na^+ is replaced by K^+. The present observation that glucose inhibited ^{45}Ca efflux even in Na^+-free medium seems incompatible with the Na^+-Ca^{2+} countertransport mechanism as the site of action for glucose. In view of the finding that neither the magnitude nor the direction of the Ca^{2+} gradient across the membrane influences the inhibition of the ^{45}Ca efflux [6] it is indeed difficult to attribute this glucose effect at all to the plasma membrane.

Previous studies of the uptake of ^{45}Ca by β-cell organelles in their normal environment have suggested that glucose stimulates the intracellular buffering of Ca^{2+} [22,23]. The reduced ^{45}Ca efflux after glucose exposure may therefore simply reflect a decreased cytosolic activity of the ion. The concept that glucose can lower cytoplasmic Ca^{2+} has recently obtained considerable support from the observation that glucose under certain conditions has a paradoxically inhibitory action on insulin secretion [24]. Since the K^+ conductance of the β-cell membrane appears to be essentially under the control of cytosolic Ca^{2+} [25,26], the physiological significance of a glucose-induced reduction of this calcium activity may well be the initiation of depolarization.

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