

Nutrient insulin secretagogues decrease $^{45}\text{Ca}^{2+}$ efflux from islet cells by a mechanism other than the inhibition of the $\text{Na}^{+}\text{-Ca}^{2+}$ countertransport

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The mechanism whereby nutrient insulin secretagogues decrease $^{45}\text{Ca}^{2+}$ efflux from islet cells is controversial. It was studied with mouse islets perfused with Ca^{2+} -free solutions. In the presence of Na^{+} , glucose and ketoisocaproate inhibited $^{45}\text{Ca}^{2+}$ efflux by about 50%. Substitution of choline $^{+}$ salts for Na^{+} salts decreased the efflux rate by 45%, but did not prevent glucose from decreasing it further. Ketoisocaproate also inhibited $^{45}\text{Ca}^{2+}$ efflux, but less markedly than in an Na^{+} medium. Omission of Na^{+} decreased the efflux rate even when it was already lowered by glucose or ketoisocaproate. It is thus clear that nutrient insulin secretagogues decrease $^{45}\text{Ca}^{2+}$ efflux from islet cells by a mechanism other than the inhibition of the $\text{Na}^{+}\text{-Ca}^{2+}$ countertransport, possibly by increasing sequestration of the ion in cellular organelles.

<i>Pancreatic islet</i>	<i>Glucose</i>	<i>Ketoisocaproate</i>	<i>Calcium efflux</i>	<i>Sodium</i>	<i>Sodium-calcium countertransport</i>
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1. INTRODUCTION

Stimulus-secretion coupling involves profound and complex modifications of Ca^{2+} handling in pancreatic B-cells. Measurements of $^{45}\text{Ca}^{2+}$ efflux from preloaded islets have shown that glucose affects several Ca^{2+} movements [1–3]. The sustained increase in efflux observed in the presence of extracellular Ca^{2+} is commonly attributed to displacement of $^{45}\text{Ca}^{2+}$ from intracellular binding sites by entering non-radioactive Ca^{2+} [3–5]. On the other hand, 2 contradictory interpretations have been given to the sustained decrease in $^{45}\text{Ca}^{2+}$ efflux brought about by glucose in the absence of extracellular Ca^{2+} . This inhibition has been ascribed to suppression of an $\text{Na}^{+}\text{-Ca}^{2+}$ countertransport at the level of the plasma membrane, because glucose had little effect on $^{45}\text{Ca}^{2+}$ efflux from rat islets perfused with an Na^{+} - and Ca^{2+} -deficient medium [6,7]. Such a mechanism could contribute to a rise in cytoplasmic Ca^{2+} concentration in B-cells. However, it has been reported recently that glucose remains able to in-

hibit $^{45}\text{Ca}^{2+}$ efflux from *ob/ob* mouse islets in an Na^{+} -free medium, provided K^{+} salts are used as substitutes for Na^{+} salts [8]. This observation was interpreted as evidence for the hypothesis that glucose promotes Ca^{2+} sequestration in cellular organelles, thereby leading to a decrease in cytoplasmic free Ca^{2+} and a secondary fall in $^{45}\text{Ca}^{2+}$ efflux. To such an effect of glucose has also been attributed the decrease in K^{+} permeability [9], that underlies the depolarization of the B-cell membrane (review [10]).

Two reasons, however, may severely limit the significance of the above observations [8]. First, the effect of glucose could be detected only in the presence of a K^{+} concentration which reverses the membrane potential of B-cells [11]. Second, recent electrophysiological experiments suggest that certain ionic permeabilities are abnormal in the B-cell membrane of *ob/ob* mice [12]. We have thus used normal NMRI mice to investigate whether an inhibition of the $\text{Na}^{+}\text{-Ca}^{2+}$ countertransport accounts for the changes in $^{45}\text{Ca}^{2+}$ efflux induced by nutrient insulin secretagogues.

2. MATERIALS AND METHODS

The perfusion technique used to monitor the efflux of $^{45}\text{Ca}^{2+}$ from preloaded islets has been detailed in [13,14]. The islets were isolated by collagenase digestion of the pancreas of fed female NMRI mice. The medium used contained 120 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl_2 , 1.2 mM MgCl_2 and 24 mM NaHCO_3 . It was gassed with O_2/CO_2 (94/6%) to maintain pH at 7.4, and was supplemented with 10 mM glucose and 1 mg bovine serum albumin/ml. Loading of the islets with $^{45}\text{Ca}^{2+}$ was carried out for 90 min in the same medium containing 15 mM glucose and supplemented with 10 mM Hepes. $^{45}\text{Ca}^{2+}$ efflux was measured in Ca^{2+} -free solutions supplemented with 10 μM atropine (even when Na^+ was present). For most experiments, CaCl_2 was simply replaced by MgCl_2 and total Ca^{2+} was less than 20 μM . Nominally Na^+ -free solutions were prepared by substituting choline chloride and choline bicarbonate for NaCl and NaHCO_3 , except in one series of experiments where NaCl was isoosmotically replaced by sucrose. When EGTA was added to a Na^+ -free medium, pH was readjusted using KOH and the concentration of KCl decreased according-

ly. Choline bicarbonate and EGTA were obtained from Sigma (St. Louis, USA), Na-ketoisocaproate from Aldrich (Brussels) and all other reagents from Merck (Darmstadt, FRG).

3. RESULTS

Addition of glucose to a Ca^{2+} -free medium containing Na^+ was followed by a sustained decrease in $^{45}\text{Ca}^{2+}$ efflux from islet cells (fig.1). This inhibition was slightly less marked with 7 mM glucose (40%) than with 15 mM glucose (50%). If the same experiments were carried out in an Na^+ -free, choline $^+$ medium, the rate of $^{45}\text{Ca}^{2+}$ efflux was already 45% lower in the absence of glucose. However, a further marked (45–50%) and sustained decrease occurred upon addition of 7 or 15 mM glucose. Withdrawal of glucose resulted in an important acceleration of $^{45}\text{Ca}^{2+}$ efflux, which followed a similar time-course in the presence or absence of Na^+ (fig.1).

The inhibition of $^{45}\text{Ca}^{2+}$ efflux by 7 mM glucose was also observed if Na^+ was not completely replaced by choline (12 mM residual Na^+) (not shown). Addition of 0.5 mM EGTA to the Ca^{2+} - Na^+ -free medium further decreased the ef-

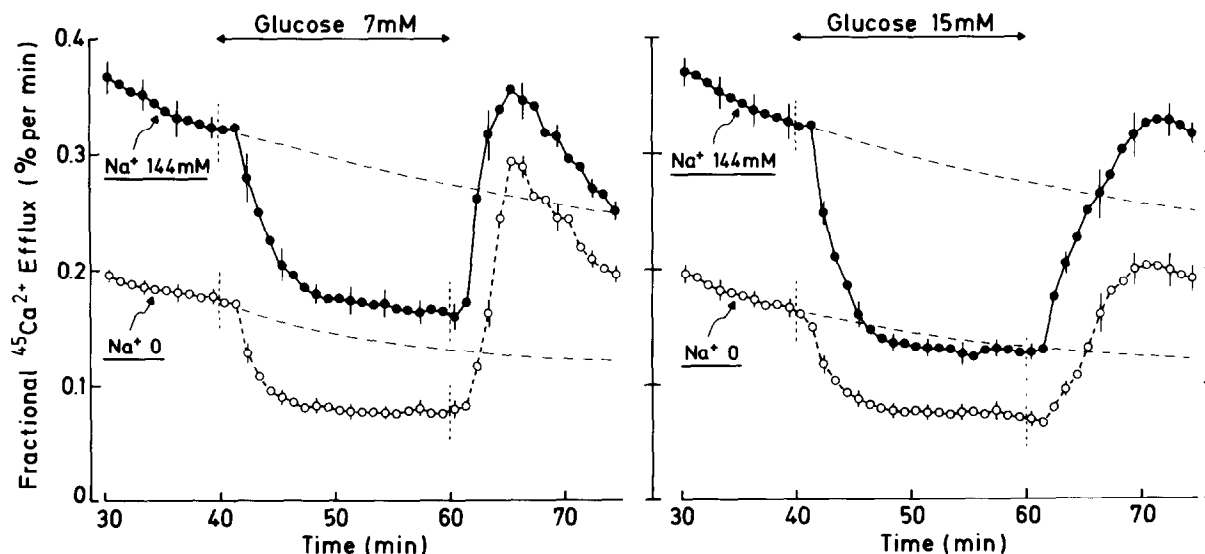


Fig.1. Effects of glucose on $^{45}\text{Ca}^{2+}$ efflux from mouse islets perfused with a Ca^{2+} -free medium (CaCl_2 being replaced by MgCl_2). Experiments were started in the absence of glucose, which was added, at the indicated concentrations, between 40 and 60 min. In two series, Na^+ salts were replaced by choline $^+$ salts. Control experiments without addition of glucose are shown by the broken lines. Values are means $\pm$ SE for 5 experiments.

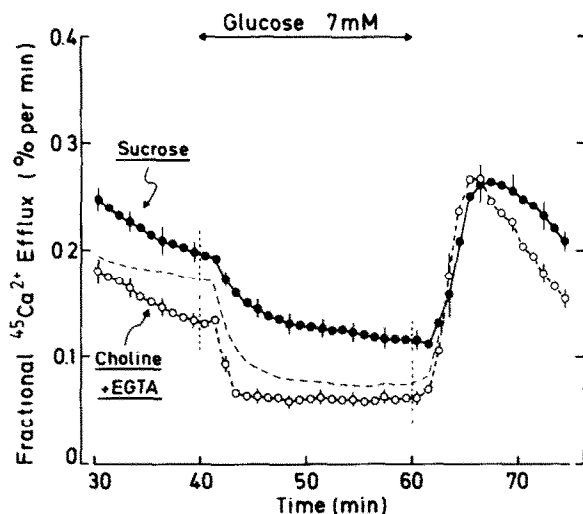


Fig.2. Effects of glucose on $^{45}\text{Ca}^{2+}$ efflux from mouse islets perfused with a Ca^{2+} - and Na^{+} -free medium. In one series (●), CaCl_2 was replaced by MgCl_2 , NaHCO_3 by choline bicarbonate and NaCl by sucrose. In the other series (○), Na^{+} salts were replaced by choline $^{+}$ salts, CaCl_2 was simply omitted, but the medium was supplemented with 0.5 mM EGTA. The broken line illustrates the same experiments as in fig.1, left panel.

Values are means $\pm$ SE for 4 experiments.

flux rate of $^{45}\text{Ca}^{2+}$, but did not prevent the inhibitory effect of glucose (fig.2). On the other hand, if sucrose was used as substitute for NaCl , the control efflux rate was less markedly decreased than with choline $^{+}$, and the inhibitory effect of glucose was less pronounced, though still unambiguously present (fig.2).

Addition of ketoisocaproate (KIC) to a Ca^{2+} -free medium containing Na^{+} was followed by a marked decrease in $^{45}\text{Ca}^{2+}$ efflux, which was slightly more rapid, but less sustained than that produced by glucose (fig.3). The amplitude of the inhibition was similar with 5 or 10 mM KIC, but the rebound increase occurring upon withdrawal of the substance displayed a consistent biphasic time course only with 10 mM KIC. In an Na^{+} -free, choline $^{+}$ -medium, KIC also inhibited $^{45}\text{Ca}^{2+}$ efflux from islet cells. Though the effect was transient, particularly with 5 mM KIC, removal of the substance from the perfusion medium resulted in a marked acceleration of $^{45}\text{Ca}^{2+}$ efflux (fig.3).

Replacement of Na^{+} by choline $^{+}$ in a glucose-free medium reversibly decreased $^{45}\text{Ca}^{2+}$ efflux by approx. 55% (fig.4). This inhibitory effect persisted when the efflux rate was already lowered by glucose or by KIC. However, its magnitude was

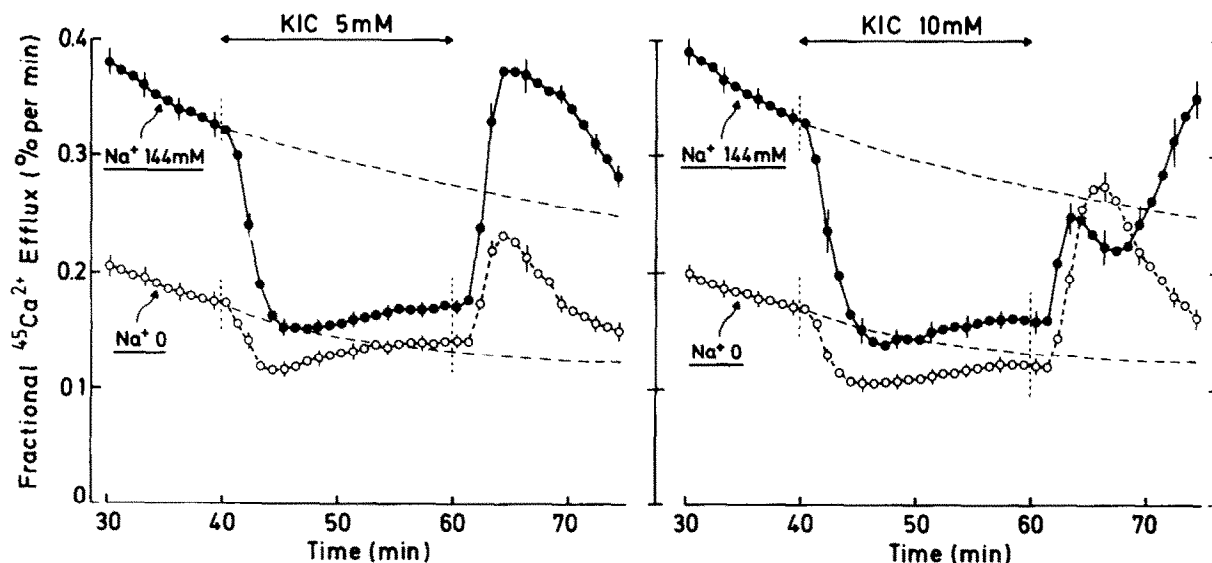


Fig.3. Effects of ketoisocaproate (KIC) on $^{45}\text{Ca}^{2+}$ efflux from mouse islets perfused with a Ca^{2+} -free medium (CaCl_2 being replaced by MgCl_2). Experiments were carried out in the absence of glucose and KIC was added, at the indicated concentrations, between 40 and 60 min. In two series, Na^{+} salts were replaced by choline $^{+}$ salts. Control experiments without addition of KIC are shown by the broken lines. Values are means $\pm$ SE for 5 experiments.

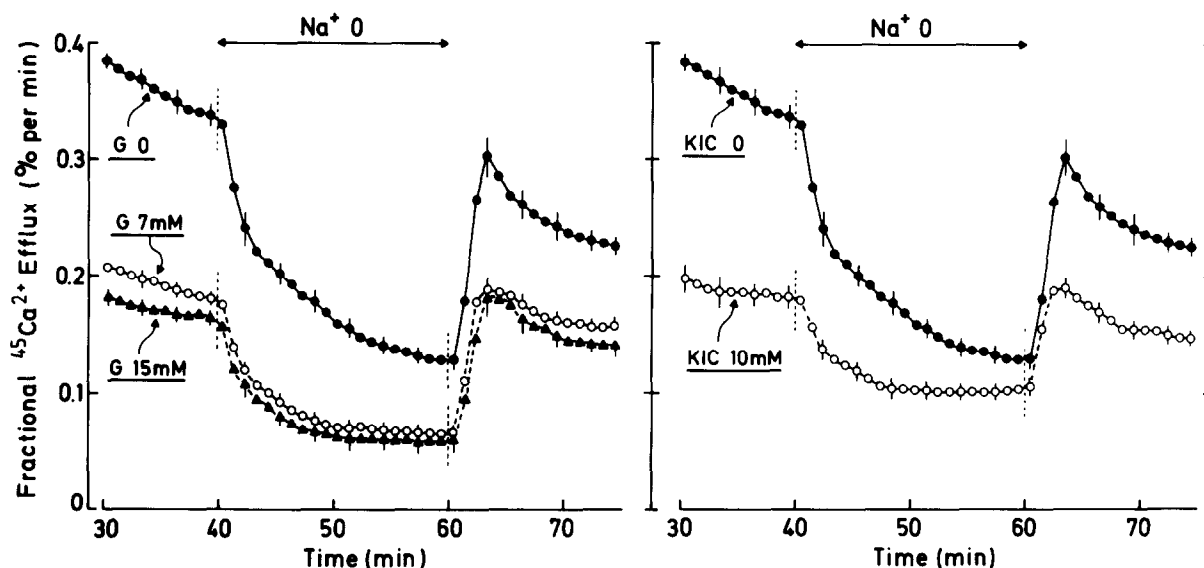


Fig.4. Effects of Na^+ omission on $^{45}\text{Ca}^{2+}$ efflux from mouse islets perfused with a Ca^{2+} -free medium (CaCl_2 being replaced by MgCl_2). Experiments were carried out in the presence of the indicated concentrations of glucose or KIC. Na^+ salts were replaced by choline $^+$ salts between 40 and 60 min. Values are means $\pm$ SE for 5 experiments.

smaller (40%) in the presence of KIC than in the presence of glucose (55%) (fig.4).

4. DISCUSSION

This study was carried out entirely in the absence of extracellular Ca^{2+} to avoid $^{40}\text{Ca}^{2+}$ - $^{45}\text{Ca}^{2+}$ exchanges. It first shows that in normal mice, as in *ob/ob* mice [8] or in rats [3,6,15], $^{45}\text{Ca}^{2+}$ efflux from islet cells depends on extracellular Na^+ . As in rats [15], an Na^+ - Ca^{2+} countertransport may account for about half of the efflux rate under basal conditions.

Omission of extracellular Na^+ inhibited $^{45}\text{Ca}^{2+}$ efflux not only in the absence of glucose, but also when the efflux rate was already lowered by glucose. It is thus clear that the Na^+ - Ca^{2+} countertransport still functions in the presence of glucose. Addition of glucose inhibited $^{45}\text{Ca}^{2+}$ efflux not only in the presence of Na^+ , but also when the efflux rate was already lowered by Na^+ omission. It is thus clear that the inhibitory effect of glucose persists when the Na^+ - Ca^{2+} countertransport is not operative. Taken together, these observations permit one to conclude that glucose inhibits $^{45}\text{Ca}^{2+}$ efflux from islet cells by another mechanism than an inhibition of the Na^+ - Ca^{2+} countertransport. It is

not possible, however, to exclude that glucose also has some influence on this countertransport. Thus, although the relative inhibitory effect of the sugar was similar in the absence or presence of Na^+ , its absolute magnitude was smaller in the Na^+ -free medium. Similarly, the relative inhibition due to Na^+ withdrawal was not affected by glucose, but the absolute decrease in the efflux rate was smaller in the presence of glucose.

Previous studies [6-8] concluded that glucose is unable to inhibit $^{45}\text{Ca}^{2+}$ efflux from islet cells when most NaCl has been replaced by sucrose or choline chloride. The reasons for the discrepancy with the present study are unclear. It is possible that earlier negative results are due to the use of sucrose as substitute for NaCl [6,7], since the effect of glucose is less clear in such solutions of low ionic strength, from which Cl^- is also almost absent.

In normal solutions, KIC and glucose produce very similar changes in ionic fluxes and membrane potential in B-cells [16,17]. Like glucose, KIC was also able to inhibit $^{45}\text{Ca}^{2+}$ efflux when the Na^+ - Ca^{2+} countertransport was not functioning. However, its effect was not sustained and much smaller than in the presence of Na^+ . This could be due to the small amount of Na^+ introduced in the

solution as Na-ketoisocaproate, to an impairment of KIC metabolism in low- Na^+ solutions or to the intracellular $^{45}\text{Ca}^{2+}$ redistribution induced by KIC [18].

If glucose does not inhibit the Na^+ - Ca^{2+} countertransport, how does it decrease $^{45}\text{Ca}^{2+}$ efflux? Our results are compatible with 2 mechanisms. First, the activity of the Ca^{2+} -extrusion pump present in the plasma membrane [19] could be decreased during B-cell stimulation by nutrient secretagogues. However, the most recent evidence, admittedly obtained with broken cells [20], argues against this interpretation. Second, glucose could promote Ca^{2+} sequestration by cellular organelles. This interpretation [8] is supported by the demonstration, with the fluorescent indicator quin2, that glucose lowers free Ca^{2+} in *ob/ob* mouse islet cells when Ca^{2+} influx is not possible [21]. One must emphasize, however, that even if such a fall in cytoplasmic Ca^{2+} occurred in normal B-cells, it would not necessarily cause the decrease in K^+ permeability and, hence, the depolarization of the B-cell membrane, as sometimes postulated [8,9]. Thus, recent patch clamp recordings have shown that the glucose-sensitive K^+ channels, which determine K^+ permeability of resting B-cells [22], are not Ca^{2+} -sensitive [23].

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