Glucose inhibits insulin release induced by Na^+ mobilization of intracellular calcium

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$^{45}Ca^{2+}$ incorporated in response to glucose was selectively mobilized from the β-cell-rich pancreatic islets of ob/ob-mice after raising the intracellular Na^+ by removal of K^+ or addition of ouabain or veratridine. Also studies of insulin release indicated opposite effects of glucose and Na^+ on the intracellular sequestration of calcium. The fact that glucose inhibits insulin release induced by raised intracellular Na^+ indicates that this sugar can lower the cytoplasmic $[Ca^{2+}]$. The concept of a dual action of glucose on the cytoplasmic $[Ca^{2+}]$. The concept of a dual action of glucose on the cytoplasmic $[Ca^{2+}]$ might well explain previous observations of an inhibitory component in the glucose action on the $^{45}Ca^{2+}$ efflux.

Insulin release *Pancreatic islet* *Ca^{2+} mobilisation* *K^+ removal* *Glucose* *Na^+ mobilization*

1. INTRODUCTION

Insulin release, like other secretory processes, is activated by an increase of cytosolic Ca^{2+}. The mechanism involved in the regulation of this Ca^{2+} has been the subject of extensive investigation. By monitoring the ^{45}Ca washout from preloaded islets it has been possible to demonstrate two distinct actions of glucose on the Ca^{2+} movements in terms of a rapid inhibition masked by a secondary rise of the ^{45}Ca efflux [1–4]. Whereas it is generally accepted that the stimulatory component reflects intracellular $^{40}Ca/^{45}Ca$ exchange due to increased influx of Ca^{2+} through voltage-dependent channels, there is a major divergence of opinion about the mechanisms involved in the glucose inhibition of ^{45}Ca efflux. This inhibition has been attributed either to suppression of the active extrusion of Ca^{2+} across the plasma membrane [3,4] or to increased sequestration of the ion in organelles [1,5,6]. It is obvious that the two alternatives are fundamentally different in having opposite effects on the cytosolic $[Ca^{2+}]$. If glucose decreases ^{45}Ca efflux by reducing the activity of cytosolic Ca^{2+}, it should even be possible to predict conditions where glucose inhibits insulin release. This communication provides evidence for such an apparently paradoxical action of glucose on the release of insulin induced by Na^+ mobilization of intracellular calcium.

2. MATERIALS AND METHODS

Pancreatic islets were obtained by microdissection after starving ob/ob-mice from a non-inbred local colony overnight. These islets contain >90% β-cells which respond appropriately to glucose and other stimulators of insulin release [7,8]. The basal medium used for the isolation of the islets and the studies of their ^{45}Ca handling and insulin release was a Hepes buffer (pH 7.4) physiologically balanced in cations with Cl^- as the sole anion [9]. The dynamics of ^{45}Ca efflux and insulin release were recorded at 37°C with parallel perifusion of islets from the same animal [10] with Ca^{2+}-deficient medium supplemented with 0.5 mM EGTA and 1 mg albumin/ml. During the perifusion, the Na^+ content of the β-cells was increased either by substitution of K^+ iso-osmotically with 5.9 mM choline$^+$ or addition of 1 mM ouabain or 100 μM veratridine. In the radioactive tracer studies the perifusion was preceded by loading for 90 min with 1.28 mM $^{45}Ca^{2+}$ in the presence and absence of 20 mM glucose. The perifused islets were freeze-dried overnight and weighed on a quartz fiber balance. ^{45}Ca was measured by liquid scintillation

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counting and insulin assayed radioimmunologically using crystalline mouse insulin as reference.

3. RESULTS

The procedures for increasing the intracellular Na^+ content mobilized intracellular calcium as indicated from a marked stimulation of the ^{45}Ca efflux when the islets had been loaded with the radioactive isotope in the presence of glucose. The significance of including glucose in the loading medium for the subsequent stimulation of the ^{45}Ca efflux obtained by raising the intracellular Na^+ is illustrated in fig.1. It is evident that the removal of K^+ results in a selective mobilization of the ^{45}Ca incorporated in response to glucose (panels A,B) and that this effect is dependent on the presence of Na^+ in the perifusion medium (panel C). In table 1 the procedures for increasing the intracellular Na^+ have been compared with regard to their ability to stimulate the efflux of ^{45}Ca after 20 min. At this time, inhibition of the Na^+ pump by removal of K^+ or addition of ouabain resulted in more pronounced effects than those obtained with veratridine. Nevertheless, the latter agent was at least as effective as a releaser of the calcium incorporated in response to glucose in exerting a more sustained effect (not shown).

The presence of glucose was important not only for the subsequent stimulation of the ^{45}Ca efflux in response to K^+ removal, but also for the possibilities of stimulating insulin release in a Ca^{2+}-deficient medium (fig.2). The increase of the intracellular Na^+ was associated with a pronounced transitory stimulation of insulin release provided that glucose was present during the period preceding the omission of K^+ (panels A,B). However, when present during the K^+ removal, glucose

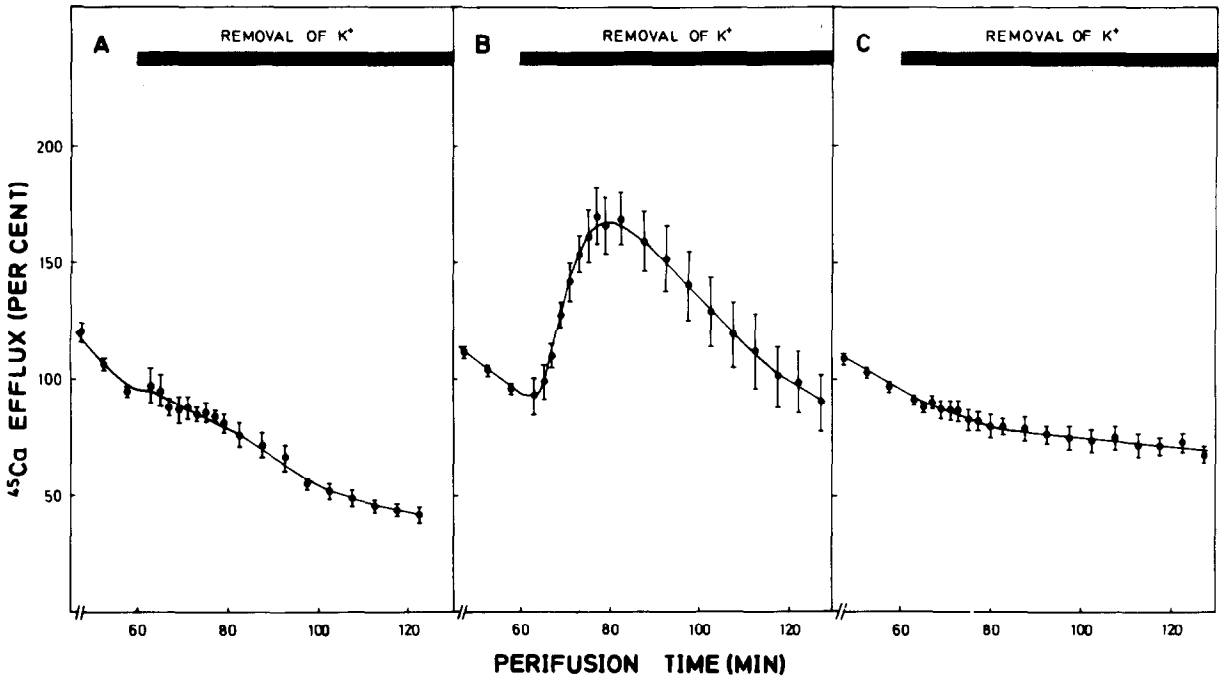

Fig.1. Effects of K^+ removal on the efflux of radioactivity into Ca^{2+}-deficient medium from islets labelled with ^{45}Ca in the presence and absence of glucose. The islets were labelled for 90 min with ^{45}Ca and perifused with a glucose-free medium deficient in Ca^{2+} and supplemented with 0.5 mM EGTA. K^+ was absent from the perifusion medium during the periods indicated by the horizontal black bars: (A) islets labelled with ^{45}Ca in the absence of glucose; (B) islets labelled in the presence of 20 mM glucose; (C) islets labelled in the presence of 20 mM glucose and perifused with medium lacking Na^+. The ^{45}Ca efflux has been given as a percentage of that noted in the individual experiment during the 10 min period preceding the removal of K^+. Mean values ± SEM for 4 expt.

Table 1

Effects of various procedures for raising intracellular Na^+ on the efflux of radioactivity from islets labelled with ^{45}Ca in the presence and absence of glucose

Procedure for raising intracellular Na^+	Changes of ^{45}Ca efflux (%)	
	Labelled at 0 mM glucose	Labelled at 20 mM glucose
Removal of K^+	−14 ± 6	+73 ± 12
Ouabain (1 mM)	−13 ± 15	+80 ± 4
Veratridine (100 μM)	−23 ± 5	+38 ± 9

The islets were labelled for 90 min with ^{45}Ca with and without the addition of 20 mM glucose and perifused with glucose-free medium deficient in Ca^{2+} and supplemented with 0.5 mM EGTA. After 60 min perifusion, the intracellular content of Na^+ was increased either by removal of K^+ or addition of 1 mM ouabain or 100 μM veratridine. The table indicates the efflux of ^{45}Ca after 80 min perifusion expressed as a percentage of that noted immediately before raising the intracellular Na^+; mean values ± SEM for 4–5 expt

acted as a potent inhibitor of the Na^+-induced insulin release (panel C).

4. DISCUSSION

Removal of extracellular K^+ [11], like the addition of ouabain or veratridine [12], induces a marked accumulation of intracellular Na^+ in isolated islets. Although different explanations have been proposed to account for the way in which glucose affects the Na^+ content in the pancreatic β-cells [12,13], there are no reasons to believe that glucose interferes with the intracellular accumulation of Na^+ obtained when the Na^+ pump is inhibited by removal of K^+. The removal of K^+ has been reported to be even more effective in stimulating the net uptake of $^{22}Na^+$ into rat islets in the presence of glucose [12].

A rise of intracellular Na^+ results in increased efflux of ^{45}Ca from preloaded rat islets even with a perifusion medium deficient in Ca^{2+} [14]. That intracellular Na^+ effectively mobilizes calcium from

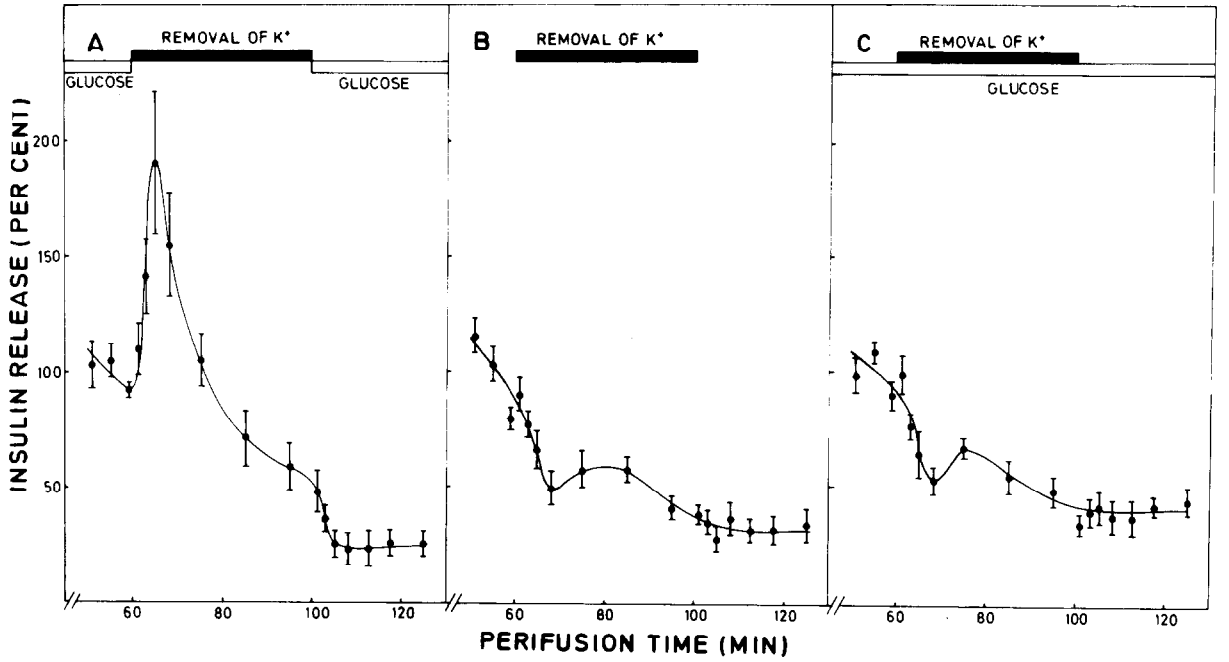

Fig.2. Effects of K^+ removal on insulin release in Ca^{2+}-deficient medium with and without added glucose. After 45 min preincubation in the presence of 1.28 mM Ca^{2+} and 3 mM glucose, the islets were perifused with Ca^{2+}-deficient medium containing 0.5 mM EGTA and 1 mg albumin/ml. K^+ was absent from the perifusion medium during the periods indicated by the horizontal black bars. The open bars indicate the periods when the perifusion medium was supplemented with 20 mM glucose. Insulin release has been given as a percentage of that noted in the individual experiment during the 10 min period preceding the removal of K^+. Mean values ± SEM for 5–7 expt.

intracellular stores is further evidenced by a pronounced stimulation of the ^{45}Ca efflux from pancreatic islets rich in β-cells whether cytoplasmic $[K^+]$ is maintained (facilitation of Na^+ influx by veratridine) or reduced (inhibition of the Na^+ pump by ouabain or K^+ removal). The mobilization of Ca^{2+} can be expected to result in enhanced concentrations of this cation in the submembrane space, a prerequisite for initiation of the insulin secretory process. Indeed, this study extends observations of the effects of increased intracellular Na^+ made with veratridine or ouabain [15,16] in demonstrating stimulation of insulin release in the absence of extracellular Ca^{2+} also after removal of K^+.

The increase of intracellular Na^+ resulted in selective mobilization of the ^{45}Ca incorporated in response to glucose. Also the outcome of the insulin release studies indicates opposite effects of glucose and Na^+ on the intracellular sequestration of calcium. Previous exposure to glucose was a prerequisite for the stimulation of insulin release obtained by the removal of K^+. Moreover, the stimulation of insulin release was suppressed in the presence of glucose. It is implicit from the observation of an inhibitory action of glucose on the insulin release induced by raised intracellular Na^+ that the glucose exposure can lower the cytoplasmic $[Ca^{2+}]$ in the β-cells. This effect might well explain the observations [1–4] of an inhibitory component in the glucose action on ^{45}Ca efflux.

Up to now, glucose inhibition of insulin release has escaped detection in normally being masked by a more pronounced stimulatory action associated with the depolarization of the β-cells with the subsequent influx of Ca^{2+}. Since the K^+ conductance of the β-cell membrane appears to be essentially under the control of the cytosolic Ca^{2+} [17,18], the reduction of this calcium activity may well be an initiating factor in the depolarizing effect of glucose. In contradiction to the stimulatory component, the inhibitory one can be expected to become less pronounced during prolonged exposure to glucose due to a limited capacity of the intracellular systems for sequestration of calcium. The concept of the dual glucose action on insulin release might consequently explain heretofore unexplained phenomena such as the appearance of a slowly increasing second phase and the fact that the secretory response can be improved by preexposure to glucose [5,6].

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