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ACTIVATION, BUT NOT INHIBITION, BY GLUCOSE OF Ca^{2+}-DEPENDENT K^+ PERMEABILITY IN THE RAT PANCREATIC B-CELL

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The effect of glucose on the Ca^{2+}-activated K^+ permeability in pancreatic islet cells was investigated by measuring the rate of ^{86}Rb efflux, ^{45}Ca efflux and insulin release from perifused rat pancreatic islets exposed to step-wise increased in glucose concentration. When the glucose concentration was raised from intermediate (8.3 or 11.1 mM) to higher values, a rapid and sustained increase in ^{86}Rb outflow, ^{45}Ca outflow and insulin release was observed. Likewise, in the presence of 8.3 or 16.7 mM glucose, tolbutamide increased ^{86}Rb and ^{45}Ca efflux, as well as insulin release. In the two series of experiments, a tight correlation was found between the magnitude of the changes in ^{86}Rb and ^{45}Ca outflow, respectively. It is concluded that, at variance with current ideas, glucose does not inhibit the response to cytosolic Ca^{2+} of the Ca^{2+}-sensitive modality of K^+ extrusion. On the contrary, as a result of its effect upon Ca^{2+} handling, glucose stimulates the Ca^{2+}-activated K^+ permeability.

Introduction

When stimulated by glucose, the insulin-producing pancreatic B-cell displays bioelectrical activity [1], which has been implicated in the process of insulin release [1–5]. At intermediate glucose concentrations (8.3 to 11.1 mM), the pattern of bioelectrical activity is characterized by the regular alternation of depolarized phases with bursts of spikes and silent polarized periods [2]. This oscillatory pattern is lost at high glucose concentrations (16.7 to 27.8 mM), glucose now provoking a permanent depolarization and continuous spiking [2]. Changes in the permeability to K^+ of the plasma membrane apparently play a critical role in the regulation of electrical activity [6–8]. On one hand, a glucose-induced decrease in K^+ permeability with subsequent gating of voltage-sensitive Ca^{2+} channels is held responsible for the depolarization at the onset of each burst of spike [8–10]. On the other hand, the repolarization phenomenon ending each burst is often attributed to the intracellular accumulation of Ca^{2+} during the burst, eventually leading to activation of a Ca^{2+}-sensitive K^+ permeability [11–15]. Both electrical and radioisotopic data support the existence, in islet cells, of such a Ca^{2+}-sensitive modality of K^+ extrusion [11–15]. It was recently proposed that the permanent depolarization seen at high glucose concentrations reflects the capacity of glucose to prevent the activation by intracellular Ca^{2+} of such a K^+ permeability [13,16–18]. The effect of an increase in the glucose concentration from an intermediate to a high value on ^{86}Rb efflux suggests, however, that high glucose concentrations activate rather than inhibit the Ca^{2+}-dependent K^+ permeability. Thus, a rise in the glucose concentration from 8.3 to 11.1 or 16.7 mM increases rather than inhibits the rate of ^{86}Rb efflux from perifused islets [19]. Such a rise is sustained [20] and suppressed in the absence of extracellular Ca^{2+} [19,21]. It is inhibited by a low concentration of quinine (5

μM) and unaffected by tetraethylammonium, suggesting that it reflects the activation of a Ca^{2+}-rather than a voltage-sensitive K^+ channel [20]. A similar increase in ^{86}Rb efflux is observed when islets perifused at an intermediate glucose concentration are suddenly exposed to other insulin secretagogues, e.g. the hypoglycemic sulfonylurea tolbutamide [22,23]. In the present work, we have further characterized the sensitivity towards glucose of the Ca^{2+}-activated K^+ permeability, by establishing the relationship between Ca^{2+} inflow and ^{86}Rb outflow, respectively, in response to step-wise increases in glucose concentration from intermediate to higher values and in response to tolbutamide.

Methods

All experiments were performed with islets isolated by the collagenase technique [24] from the pancreas of fed albino rats.

The media used for incubating, washing or perifusing the islets consisted of a Krebs-Ringer bicarbonate-buffered solution supplemented with 0.5% (w/v) dialyzed albumin (Fraction V; Sigma Chemical Company, St. Louis, MO) and equilibrated against a mixture of O_2 (95%) and CO_2 (5%). Some media contained no $CaCl_2$ and were enriched with 0.5 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N'*-tetraacetic acid (EGTA). The media also contained, as required, glucose and tolbutamide (Hoechst, Frankfurt, F.R.G.).

The method used for the measurement of ^{86}Rb efflux, ^{45}Ca efflux and insulin release from perifused islets has been described elsewhere [25,26]. Briefly, groups of 100 islets each were incubated for 60 min in the presence of 16.7 mM glucose and either ^{86}Rb (0.3–0.5 mM; 100 μCi/ml) or ^{45}Ca (1.12 mM; 200 μCi/ml). After incubation, the islets were washed three times and then placed in a perifusion chamber. The perifusate was delivered at a constant rate (1.0 ml/min). From the 31st to the 90th min, the effluent was collected continuously over successive periods of 1 min each. An aliquot of the effluent (0.4 ml) was used for scintillation counting while the remainder was stored at $-20°C$ for insulin assay. At the end of the perifusion, the radioactive content of the islets was also determined. The efflux of ^{86}Rb and ^{45}Ca (cpm per min) was expressed as a fractional outflow rate (% of instantaneous islet content per min: F.O.R.). The validity of ^{86}Rb as a tracer for the study of K^+ handling in the islets has been assessed elsewhere [26].

All results are expressed as the mean ($\pm$ S.E.) together with the number of individual experiments (n). The magnitude of the glucose- or tolbutamide-induced increase in the rate of ^{86}Rb and ^{45}Ca efflux was estimated, in each individual experiment from the difference between the lowest value recorded just prior to or at the time when either the glucose concentration was increased or tolbutamide added to the perifusate and the highest value reached a few min later. The statistical significance of differences between data was evaluated by using the non-paired Student's t-test.

Results

A step-wise increase in the glucose concentration from 8.3 mM to 11.1, 16.7 or 27.8 mM provoked a graded increase in the rates of ^{86}Rb efflux, ^{45}Ca efflux and insulin release from islets perifused in the presence of 1 mM extracellular Ca^{2+} (Fig. 1). Such a graded response was also observed when the glucose concentration was raised from 11.1 to 16.7 or 27.8 mM (Fig. 2). In these experiments, there was a close correlation between the effects of glucose upon ^{86}Rb and ^{45}Ca efflux, respectively, whether the results were expressed as increments in the rate of efflux or as the absolute peak values reached shortly after raising the glucose concentration (Fig. 4).

In the presence of 8.3 mM glucose, tolbutamide (0.37 mM) provoked a rapid, sustained and rapidly reversible increase in ^{86}Rb efflux, ^{45}Ca efflux and insulin release (Fig. 3). When the same experiment was carried out in the presence of 16.7 mM glucose (Fig. 3), tolbutamide also increased ^{86}Rb efflux, ^{45}Ca efflux and insulin release from perifused islets, but the increases in both ^{86}Rb efflux and ^{45}Ca efflux were reduced in their magnitude ($P < 0.025$ or less). The latter experiments also differed from the former by a higher basal rate of ^{86}Rb efflux, ^{45}Ca efflux and insulin release prior to administration of tolbutamide (time 31 to 44 min). There was also a close correlation between

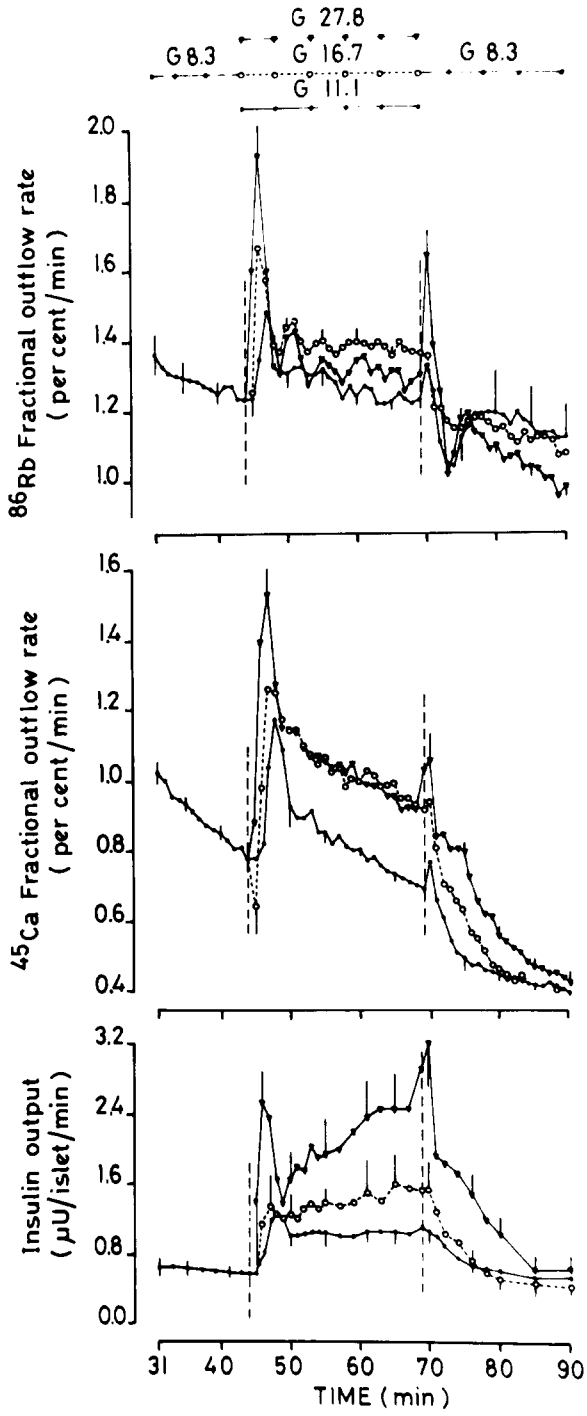


Fig. 1. Effect of a graded increase in the glucose concentration from 8.3 mM to 11.1 mM (●———●), 16.7 mM (○- - - - - -○) or 27.8 mM (▼———▼) on ^{86}Rb efflux (upper panel), ^{45}Ca efflux (middle panel) and insulin release (lower panel) from islets perifused at normal extracellular Ca^{2+} concentration (1 mM). Mean values (±S.E.) for ^{86}Rb efflux, ^{45}Ca efflux and insulin release are expressed as fractional outflow rates (F.O.R.) and refer in each case to four individual experiments.

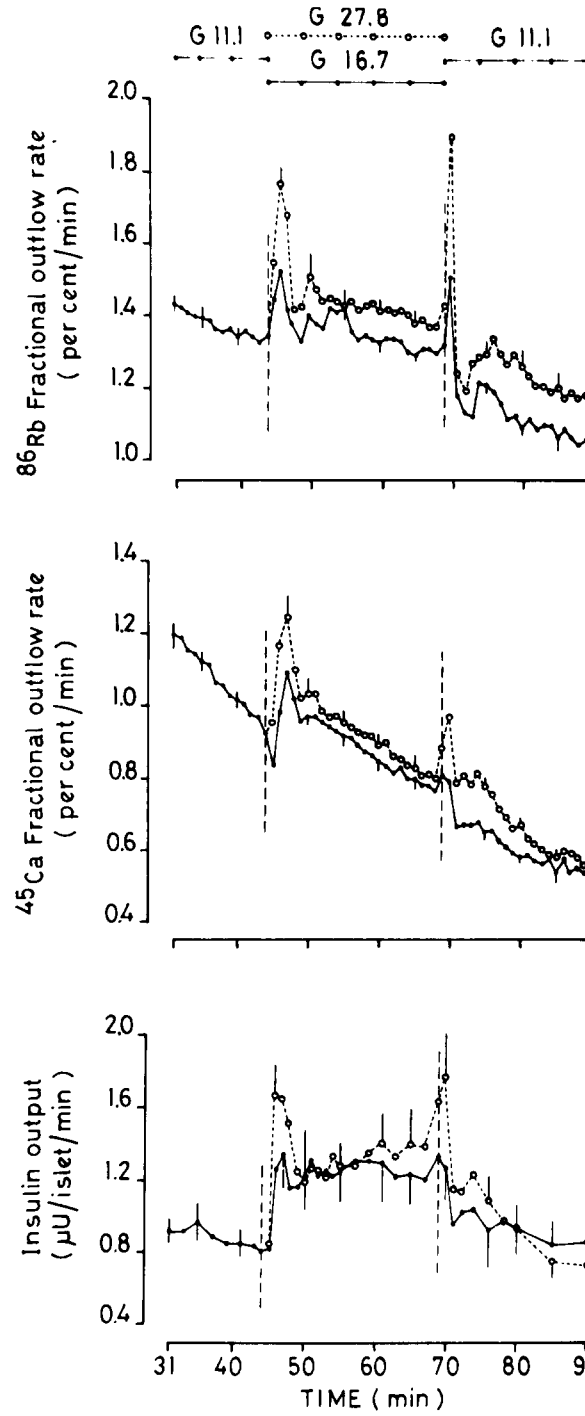
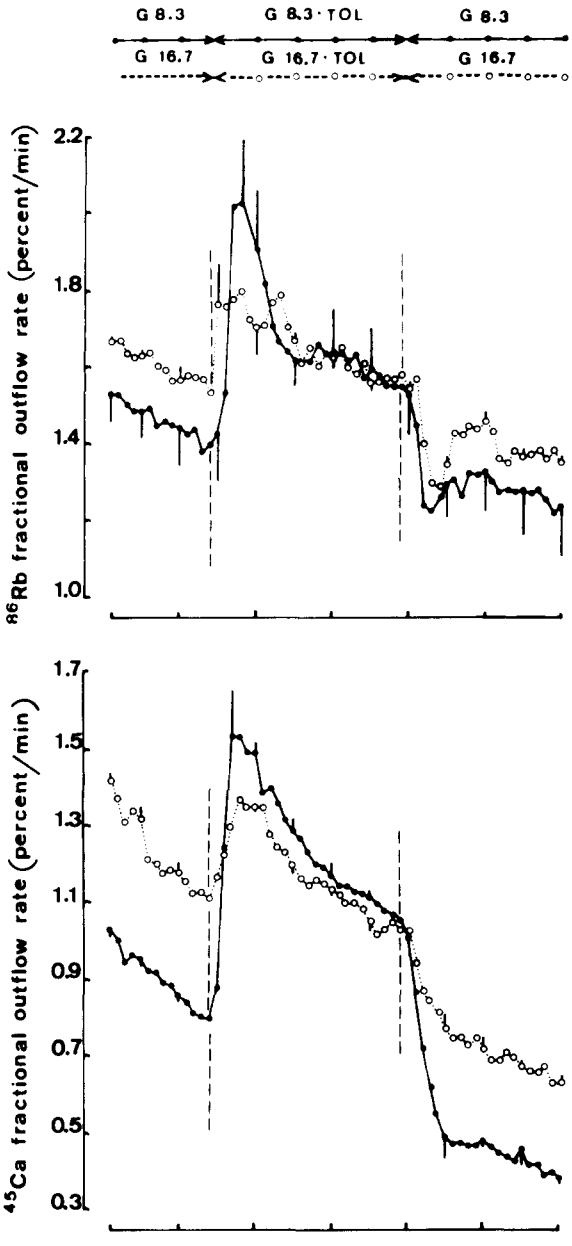


Fig. 2. Effect of an increase in the glucose concentration from 11.1 mM to 16.7 mM (●———●) or 27.8 mM (○- - - - - -○) on ^{86}Rb efflux (upper panel), ^{45}Ca efflux (middle panel) and insulin release (lower panel) from islets perifused at normal extracellular Ca^{2+} concentration (1 mM). Mean values (±S.E.) for ^{86}Rb efflux, ^{45}Ca efflux and insulin release are expressed as in Fig. 1 and refer in each case to four individual experiments.



the effects of tolbutamide upon ^{86}Rb and ^{45}Ca efflux (Fig. 4).

When the glucose concentration was raised from 16.7 to 27.8 mM, no obvious change in the rate of ^{45}Ca efflux and insulin release was noticed, whereas the rate of ^{86}Rb efflux was slightly decreased (Fig. 5).

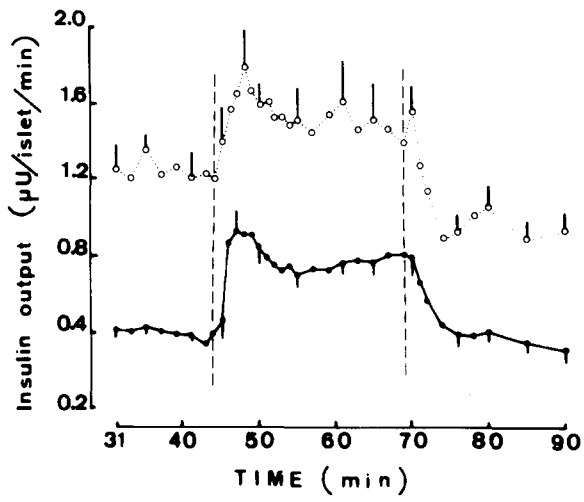


Fig. 3. Effect of tolbutamide (0.37 mM) upon ^{86}Rb efflux (upper panel), ^{45}Ca efflux (middle panel) and insulin release (lower panel) from islets perifused at normal extracellular Ca^{2+} concentration either in the presence of 8.3 mM (●———●) or 16.7 mM glucose (○- - - - -○). Mean values (±S.E.) for ^{86}Rb efflux, ^{45}Ca efflux and insulin release expressed as in Fig. 1 and refer in both cases to four individual experiments.

Discussion

The present data confirm previous observations showing that a rise in glucose concentration from intermediate to higher values [19–21] or the addition of the hypoglycemic sulfonylurea tolbutamide

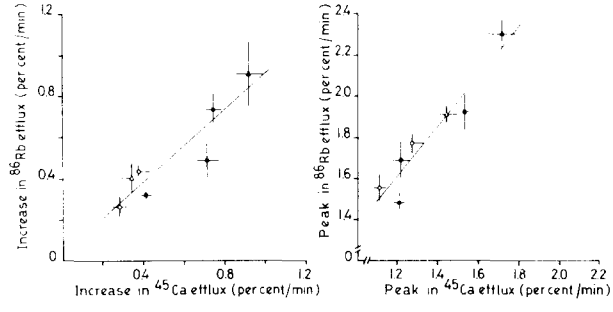


Fig. 4. Correlation between the increases above basal value (left panel) or the peak values (right panel) for the rate of ^{86}Rb efflux and ^{45}Ca efflux. Mean values (±S.E.) are taken from Figs. 1–3. Closed circles (●): the concentration of glucose was raised from 8.3 to 11.1, 16.7 or 27.8 mM. Open circles (○): the concentration of glucose was raised from 11.1 to 16.7 or 27.8 mM. Triangles: tolbutamide (0.37 mM) was added to a medium containing 8.3 mM glucose (▲, $n = 4$) or 16.7 mM glucose (△, $n = 4$). The straight lines correspond to the regression line derived from mean data.

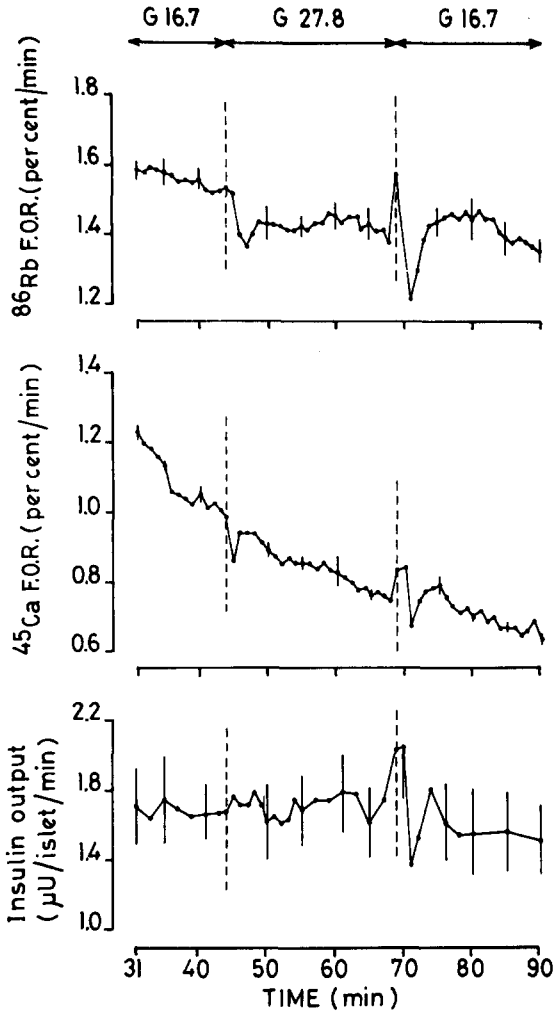


Fig. 5. Effect of a rise in the glucose concentration from 16.7 to 27.8 mM on ^{86}Rb efflux (upper panel), ^{45}Ca efflux (middle panel) and insulin release (lower panel) from islets perifused at normal extracellular Ca^{2+} concentration (1 mM). Mean values (±S.E.) for ^{86}Rb efflux, ^{45}Ca efflux and insulin release are expressed as in Fig. 1 and refer in each case to four to six individual experiments.

to islets exposed to an intermediate glucose concentration, increase the rate of ^{86}Rb efflux from perifused islets [22,23]. This increase coincides with an increase in ^{45}Ca efflux, which is known to reflect a stimulation of Ca^{2+} entry into the islet cells and to correspond to a process of Ca–Ca exchange in which influent ^{40}Ca displaces ^{45}Ca from intracellular binding sites [25,27]. The increase in ^{86}Rb efflux, observed under the present

TABLE I

RATE OF ^{86}Rb EFFLUX OVER TWO PERIODS OF TIME IN THE PRESENCE OF VARIOUS GLUCOSE CONCENTRATIONS

The coefficient of correlation (r) between ^{86}Rb fractional outflow rate and glucose concentration is shown together with its statistical significance (P).

Glucose (mM)	^{86}Rb fractional outflow rate (per cent/min)	
	30–44 min	50–68 min
8.3	1.291 ± 0.038 (16)	
11.1	1.347 ± 0.023 (8)	1.267 ± 0.038 (4)
16.7	1.548 ± 0.024 (12)	1.369 ± 0.015 (8)
27.8		1.397 ± 0.024 (14)
r	0.696	0.452
P	< 0.001	< 0.05

experimental conditions, has been proposed to result from the activation by intracellular Ca^{2+} of the Ca^{2+}-sensitive K^+ permeability [19–21]. This is supported by the present observation that no augmentation in the rate of ^{86}Rb efflux could be observed (Fig. 5) when the initial concentration of glucose was so high (16.7 mM) as to prevent any increase in ^{45}Ca efflux and insulin release in response to a further rise in glucose concentration.

Moreover, when the islets were examined under close-to-steady conditions, the fractional outflow rate of ^{86}Rb correlated positively with the glucose concentration in the 8.3 to 27.8 mM range (Table I). This behaviour is in sharp contrast with the claim made by Henquin that, under steady-state conditions, the K^+ permeability decreases, progressively albeit modestly, as a function of the glucose concentration in the 8.3 to 20.0 mM range [8].

In the present experiments, there was a significant and positive correlation between the magnitude of the increases in ^{86}Rb and ^{45}Ca outflow rates, respectively, whether they were evoked by a rise in the glucose concentration from an intermediate to a higher value or by the addition of tolbutamide to islets exposed to an intermediate glucose concentration. This close correlation suggests that the rate of ^{86}Rb efflux, under the present experimental conditions, is regulated mainly by the rate of ^{40}Ca inflow rate into the islet cells,

as reflected by the extent of ^{40}Ca-^{45}Ca exchange. With respect to such a correlation, the data obtained at different glucose concentrations or in the presence of tolbutamide apparently all belong to a homogeneous population. In other words, it appears that the dependency of ^{86}Rb efflux on the rate of Ca^{2+} inflow was unaffected by the actual concentration of glucose or presence of tolbutamide, suggesting that neither glucose nor tolbutamide modulate to any obvious extent the sensitivity towards Ca^{2+} of the Ca^{2+}-responsive K^+ permeability.

Our data may appear incompatible with the claim that glucose suppresses the increase in ^{86}Rb efflux evoked by the Ca^{2+} ionophore A23187 or other agents supposed to increase the cytosolic concentration of Ca^{2+} in the islet cells [13]. Such is not necessarily the case, since the suppressive effect of glucose, under these less physiological conditions, could be secondary to a change in Ca^{2+} handling rather than to a primary effect of glucose upon the responsiveness to Ca^{2+} of the Ca^{2+}-sensitive K^+ permeability. For instance, when the concentration of glucose is increased, the B-cell may be better able to counteract the effect of pharmacological agents upon cytosolic Ca^{2+}, e.g. by an increase in Ca^{2+}-ATPase activity resulting in a higher capacity to sequester Ca^{2+} in intracellular organelles [28].

In conclusion, the present data provide further evidence that glucose even in very high concentrations, does not inactivate the Ca^{2+}-sensitive modality of K^+ extrusion. On the contrary, stepwise increases in glucose concentration, by facilitating Ca^{2+} inflow into the B-cell, provoked parallel increases in ^{86}Rb outflow rate. It appears most unlikely, therefore, that the capacity of glucose, in high concentration, to delay or suppress the repolarisation phenomenon at the end of each burst of spikes is attributable to inactivation of the Ca^{2+}-dependent K^+ permeability. A number of alternative explanations can be visualized and each of them now requires careful examination.

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