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$Na^+ - K^+$ Pump Activity and the Glucose-Stimulated Ca^{2+}-Sensitive K^+ Permeability in the Pancreatic B-Cell

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Summary. A rise in the extracellular concentration of glucose from an intermediate to a high value changes the burst pattern of electrical activity of the pancreatic B-cell into a continuous firing, and yet activates the B-cell Ca^{2+}-sensitive K^+ permeability. The hypothesis that glucose exerts such effects by inhibiting the Na^+, K^+-ATPase was investigated. Ouabain (1 mM) mimicked the effect of 16.7 mM glucose in stimulating ^{86}Rb, ^{45}Ca outflow and insulin release from perifused rat pancreatic islets first exposed to 8.3 mM glucose. The stimulation by ouabain of ^{86}Rb outflow was reduced in the absence of extracellular Ca^{2+} and almost completely abolished in the presence of quinine, and inhibitor of the Ca^{2+}-sensitive K^+ permeability. In the presence of ouabain, a rise in the glucose concentration from 8.3 to 16.7 mM failed to stimulate ^{86}Rb outflow. However, the rise in the glucose concentration failed to inhibit ^{86}Rb influx in islet cells, while ouabain dramatically reduced ^{86}Rb influx whether in the presence of 8.3 or 16.7 mM glucose. These findings do not suggest that inhibition of the B-cell Na^+,K^+-ATPase represents the mechanism by which glucose in high concentration stimulates ^{86}Rb outflow and induces continuous electrical activity in the B-cell.

Key Words Na^+,K^+-ATPase · Ca^{2+}-activated K^+ permeability · ouabain · quinine · pancreatic B-cell · insulin release

Introduction

Changes in K^+ permeability play a major role in the mechanisms by which glucose stimulates electrical activity in the pancreatic B-cell [27]. Indeed, glucose is thought to depolarize the B-cell plasma membrane by reducing K^+ conductance leading to the gating of voltage-sensitive Ca^{2+} channels and subsequent stimulation of insulin release [6, 8, 12, 19, 31, 36]. Furthermore, it has been proposed that high concentration of glucose, may enhance electrical activity and insulin release by preventing the activation by intracellular Ca^{2+} of a Ca^{2+}-sensitive modality of K^+ extrusion [13, 29]. In the presence of an intermediate concentration of glucose (8.3 to 11.1 mM), the electrical activity displays an oscillatory pattern consisting in the regular alternation of depolarization phases with bursts of spikes and silent repolarized periods [10]. The activation of a Ca^{2+}-sensitive modality of K^+ transport may participate in the repolarization phase at the end of each burst of spikes [2–4, 13, 15, 33]. However, the view that glucose, in high concentration, may induce continuous electrical activity by inhibiting this Ca^{2+}-sensitive modality of K^+ extrusion is difficult to reconcile with recent observations which rather suggest that, as a result of its stimulatory effect upon Ca^{2+} inflow into the B-cell, glucose stimulates the Ca^{2+}-activated K^+ permeability. Thus, a rise in the glucose concentration from an intermediate to a high value stimulates rather than inhibits the efflux of ^{86}Rb (used as a tracer for K^+) from perifused islets [9]. Such a stimulation is sustained [24] and reflects the activation of a Ca^{2+}-sensitive modality of K^+ transport, it being suppressed in the absence of extracellular Ca^{2+} [9, 28], inhibited by a low concentration of quinine [23] and unaffected by tetraethylammonium [23]. Moreover, in islets stimulated either by step-wise increases in glucose concentration from intermediate to higher values or by the hypoglycemic sulfonylurea tolbutamide (in the presence of 8.3 mM glucose), there is a close correlation between stimulated Ca^{2+} inflow and ^{86}Rb outflow [24]. Last, when the islets are examined under close-to-steady conditions, the fractional outflow rate of ^{86}Rb correlates positively with the glucose concentration in the 8.3 to 27.8 mM range [24].

The aim of the present study was to examine whether an inhibition by glucose of Na^+,K^+-ATPase participates in the increase in ^{86}Rb outflow seen when the concentration of the sugar is raised from an intermediate to a high value and to investigate whether such an inhibition may account for the effect of glucose in high concentration to suppress the repolarization phenomenon at the end of each burst of spikes. The B-cell has been shown to be equipped with a ouabain-sensitive Na^+,K^+-ATPase [20, 35, 37]. It was postulated that activation of such a pump participates

in the repolarization at the end of each burst [31], so that the continuous firing seen at high glucose concentrations would imply that glucose somehow prevents the activation of the Na^+,K^+-ATPase.

Materials and Methods

All experiments were performed with islets isolated by the collagenase technique [21] from the pancreas of fed albino rats.

The media used for incubating, washing or perifusing the islets consisted of a Krebs-Ringer's bicarbonate-buffered solution having the following composition (in mM): NaCl 115, KCl 5, $CaCl_2$, 1, $MgCl_2$ 1, $NaHCO_3$ 24. The media were 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%). Some media contained no $CaCl_2$ and were enriched with 0.5 mM ethylene glycol bis-(β-aminoethylether)-N,N'-tetra-acetic acid (EGTA). The media also contained glucose, quinine and ouabain (Sigma Chemical Company), when required.

The method used for the measurement of ^{86}Rb efflux, ^{45}Ca efflux and insulin release from perifused islets has been described elsewhere [18, 22]. 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 to 0.5 mM; 100 μCi/ml) or ^{45}Ca (1.12 mM; 200 μCi/ml). After incubation, the islets were washed 3 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., *see* ref. [18]). The validity of ^{86}Rb as a tracer for the study of K^+ handling in the islets has been assessed elsewhere [26].

For the measurement of ^{86}Rb uptake, groups of 10 islets each derived from the same batch of islets were placed in polythene microcentrifuge tubes and preincubated for 30 min at 37 °C in 0.05 ml of a nonradioactive medium placed on the top of a layer of oil (Versilube F50, General Electric, Waterford, New York). The islets were then incubated for a further 5 min in 0.1 ml of the same medium containing in addition [6,6'(*n*)-^{3}H]-sucrose (1.0 mM; 20 μCi/ml) and ^{86}Rb (0.11 mM, 10 μCi/ml). The latter media also contained glucose and ouabain, when required. At the end of the incubation, the islets were separated from the incubation medium by centrifugation through the layer of oil. The net uptake of ^{86}Rb was corrected for extracellular contamination as described elsewhere [26].

All results are expressed as the mean (±SEM) together with the number of individual experiments (*n*). The statistical significance of differences between mean data was evaluated by using the nonpaired Student's *t*-test for two, and variance analysis for multisample comparison.

Results

Effect of Ouabain on ^{86}Rb, ^{45}Ca Efflux and Insulin Release in the Absence of Glucose

In the absence of glucose, 1 mM ouabain provoked a dual change in the rate of ^{86}Rb efflux from islets perifused in the presence of extracellular Ca^{2+} (Fig. 1, upper panel). It consisted of a transient fall, followed by a small but sustained increase in ^{86}Rb efflux. The removal of ouabain was followed by a decrease in ^{86}Rb outflow rate which exceeded that expected from the simple suppression of the stimulatory effect of the drug upon ^{86}Rb efflux. Such a situation is reminiscent of the hyperpolarization seen on removal of ouabain, a phenomenon attributed to reactivation of the Na^+,K^+-ATPase by intracellularly accumulated Na^+ [5, 14, 30]. Ouabain also gradually increased the rate of ^{45}Ca efflux (Fig. 1, middle panel), but failed to stimulate insulin release from the glucose-deprived islets (Fig. 1, lower panel). When the same experiments were carried out in the absence of extracellular Ca^{2+}, ouabain failed to stimulate and instead decreased ^{86}Rb outflow rate (Fig. 1, upper panel). In the absence of extracellular Ca^{2+}, ouabain also provoked a gradual increase in ^{45}Ca efflux, but this increase was of lesser magnitude than that observed in the presence of extracellular Ca^{2+} (Fig. 2, middle panel). Thus, in the absence of Ca^{2+}, the increment in ^{45}Ca efflux, above baseline value (min 41 to 44) observed during the last 10 min of perifusion in the presence of ouabain only averaged 33%

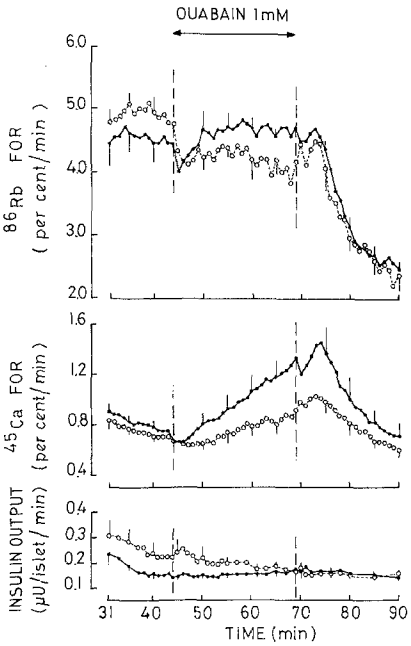

Fig. 1. Effect of 1 mM ouabain on ^{86}Rb efflux (upper panel), ^{45}Ca efflux (middle panel) and insulin release (lower panel), from glucose-deprived islets perifused either in the presence (●—●) or the absence of extracellular Ca^{2+} (o—o). Mean values (±SEM) for ^{86}Rb efflux and ^{45}Ca efflux are expressed as a fractional outflow rate and refer in each case to 4 individual experiments. Mean values (±SEM) for insulin release are expressed in μU/islet/min and refer to 4 individual experiments

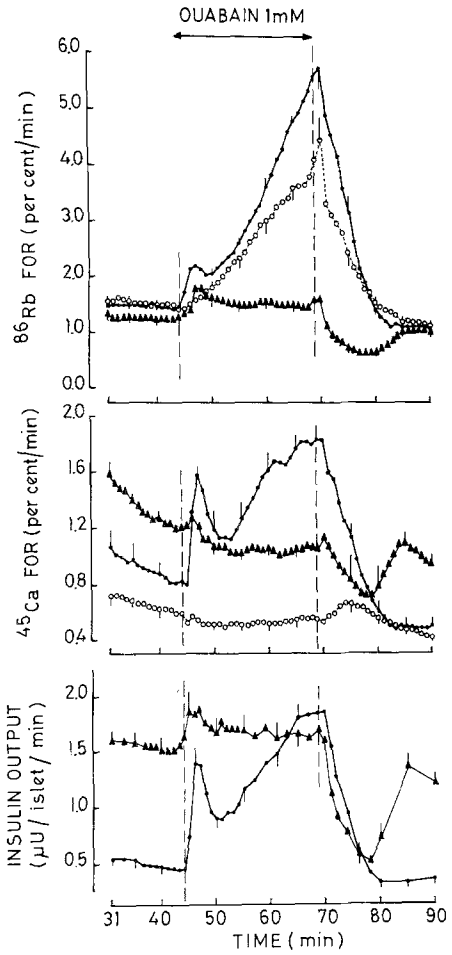

Fig. 2. Effect of 1 mM ouabain on ^{86}Rb efflux (upper panel), ^{45}Ca efflux (middle panel) and insulin release (lower panel) from islets perifused throughout in the presence of 8.3 mM glucose. Basal media contained Ca^{2+} (1 mM; ●—●), Ca^{2+} and quinine (1 mM and 100 μM, respectively; ▲—▲) or were deprived of Ca^{2+} (o---o). Same presentation as in Fig. 1; $n=4$ in each case

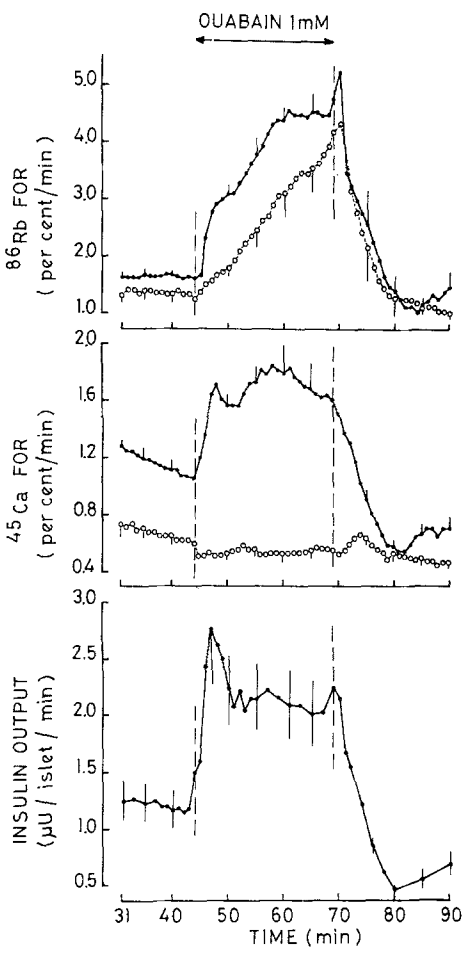

Fig. 3. Effect of 1 mM ouabain on ^{86}Rb efflux (upper panel), ^{45}Ca efflux (middle panel) and insulin release (lower panel) from islets perifused throughout in the presence of 16.7 mM glucose. Basal media contained Ca^{2+} (1 mM; ●—●), or were deprived of Ca^{2+} (o---o). Same presentation as in Fig. 1; $n=4$ in each case

of that observed at a normal extracellular Ca^{2+} concentration ($P<0.001$).

Effect of Ouabain on ^{86}Rb, ^{45}Ca Efflux and Insulin Release from Islets Perifused in the Presence of Glucose

In the presence of 8.3 mM glucose, 1 mM ouabain provoked a rapid, biphasic and important increase in ^{86}Rb efflux, ^{45}Ca efflux and insulin release from islets perifused in the presence of extracellular Ca^{2+} (Fig. 2). These increases were rapidly reversed on removal of the drug from the perifusate. When the same experiments were carried out in the absence of extracellular Ca^{2+}, the ouabain-induced increment in ^{86}Rb efflux was decreased by 37% ($P<0.01$, Fig. 2, upper panel) and the increase in ^{45}Ca efflux was completely abolished (Fig. 2, middle panel). Quinine, which is known to inhibit the Ca^{2+}-sensitive K^+ permeability [1, 4] increased the rate of ^{45}Ca efflux and insulin release ($P<0.025$ and 0.001, respectively) prior to administration of ouabain (min 31 to 44) and almost completely abolished the increases in ^{86}Rb efflux ($P<0.001$), ^{45}Ca efflux and insulin release ($P<0.001$ in both cases) otherwise evoked by the cardiotonic glycoside (Fig. 2).

When the islets were perifused in the presence of 16.7 mM glucose, ouabain also provoked a rapid, sustained and dramatic increase in ^{86}Rb efflux, ^{45}Ca efflux and insulin release (Fig. 3). In the absence of extracellular Ca^{2+}, the ouabain-induced increase in ^{86}Rb outflow from islets perifused in the presence of 16.7 mM glucose was reduced by 36% ($P<0.05$, Fig. 3, upper panel), and the increase in ^{45}Ca outflow was completely

abolished (Fig. 3, middle panel). The ouabain-induced increase in ^{86}Rb outflow was slightly but not significantly higher in the presence of 16.7 mM than 8.3 mM glucose ($P>0.1$).

Figure 4 illustrates the effect of a rise in glucose concentration from an intermediate to a high value upon ^{86}Rb efflux, ^{45}Ca efflux and insulin release from islets perifused in the absence (left panel) or presence (right panel) of ouabain. In the absence of ouabain, a rise in the glucose concentration from 8.3 to 16.7 mM provoked a biphasic, sustained and rapidly reversible increase in ^{86}Rb efflux, ^{45}Ca efflux and insulin release [24]. When the same experiments were carried out in the presence of ouabain throughout, several differences could be observed (Fig. 4, right panel). First, the rates of ^{86}Rb efflux, ^{45}Ca efflux and insulin release observed before stimulation by 16.7 mM glucose, were higher than in the absence of ouabain ($P<$ 0.005 or less). Second, in the presence of ouabain, the rise in the glucose concentration from 8.3 to 16.7 mM, instead of increasing the rate of ^{86}Rb outflow, tended to slightly decrease ^{86}Rb outflow rate. Last, the rise in the glucose concentration virtually failed to affect the rate of ^{45}Ca efflux and insulin release in the islets exposed to ouabain.

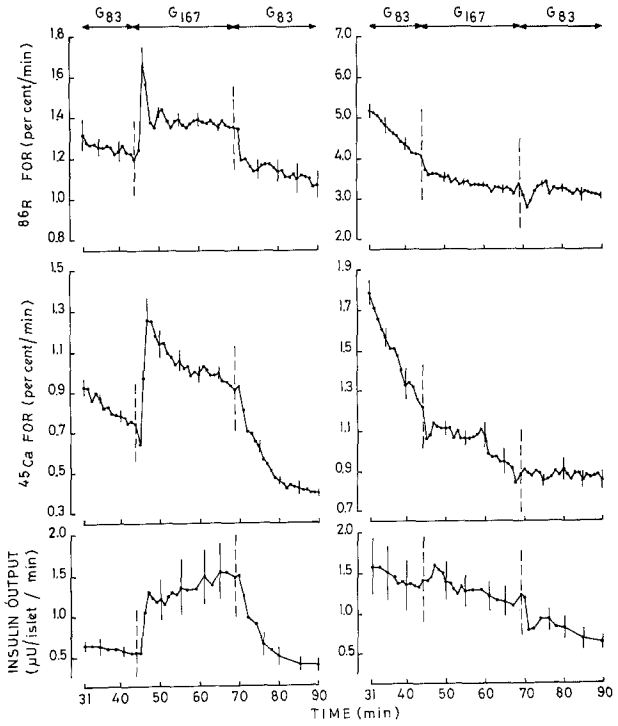

Fig. 4. Effect of a rise in the glucose concentration from 8.3 to 16.7 mM upon ^{86}Rb efflux (upper panels), ^{45}Ca efflux (middle panels) and insulin release (lower panels) from islets perifused in the absence (left panels) or presence (right panels) of 1 mM ouabain. Basal media contained Ca^{2+} (1 mM). Same presentation as in Fig. 1; $n=4$ or 6 individual experiments

Effect of Glucose and Ouabain on ^{86}Rb Influx

In order to further evaluate the effect of glucose and ouabain upon the Na^+,K^+-ATPase, ^{86}Rb uptake was measured over short-term experiments (5 min). When the islets had been preincubated during 30 min in the absence of glucose, the sugar, in a range of concentration from 0 to 16.7 mM failed to affect ^{86}Rb uptake (Table). Likewise, when the islets had been preincubated in the presence of 8.3 or 16.7 mM glucose, a rise or a decrease in the glucose concentration from 8.3 to 16.7 mM or from 16.7 to 8.3 mM, respectively, failed to affect ^{86}Rb uptake (Table). In contrast, in islets preincubated in the presence of 8.3 mM glucose, ouabain significantly decreased ^{86}Rb uptake whether the islets were incubated in the presence of 8.3 or

Table. Effect of glucose and ouabain on ^{86}Rb uptake

Experiment	Line	Preincubation[a]	Incubation[a]		^{86}Rb uptake[b]	*P*
		glucose (mM)	glucose (mM)	ouabain (mM)	pmol/islet/5 min	
A	1	0	0	Nil	52.62±3.24 (16)	
	2	0	4.2	Nil	45.56±3.19 (16)	2 *vs.* 1 NS
	3	0	8.3	Nil	45.26±2.76 (16)	3 *vs.* 2 NS
	4	0	16.7	Nil	52.69±3.56 (16)	4 *vs.* 3 NS
B	5	8.3	8.3	Nil	38.35±1.60 (77)	5 *vs.* 6<0.001
	6	8.3	8.3	1.0	22.00±1.67 (16)	6 *vs.* 8 NS
	7	8.3	16.7	Nil	40.46±1.73 (46)	7 *vs.* 5 NS
	8	8.3	16.7	1.0	20.71±1.40 (15)	8 *vs.* 7<0.001
	9	16.7	8.3	Nil	33.33±1.46 (31)	
	10	16.7	16.7	Nil	37.44±1.62 (30)	10 *vs.* 9 NS

[a] The islets were preincubated during 30 min and incubated during 5 min.
[b] Expressed as picomoles of K^+ with the same specific activity ($^{86}Rb/^{39}K$) as that of the incubation medium.

16.7 mM glucose (Table). The mean values for ^{86}Rb uptake under the latter two experimental conditions were not significantly different from one another (Table).

Discussion

In the present study we have examined whether an inhibition of the islet Na^+,K^+-ATPase as mediated by ouabain may mimic the effect of a rise in the glucose concentration from an intermediate to a high value in activating the Ca^{2+}-sensitive modality of K^+ transport and whether glucose, like ouabain, may inhibit the Na^+,K^+-ATPase.

The Mechanism of Ouabain-Stimulated ^{86}Rb Outflow

At a concentration of 1 mM, ouabain mimicked the effect of 16.7 mM glucose to stimulate ^{86}Rb outflow from islets first exposed to 8.3 mM glucose. Such a stimulation, like that evoked by 16.7 mM glucose, apparently resulted from the activation of a Ca^{2+}-sensitive modality of K^+ efflux, it being almost completely suppressed in the presence of quinine [1, 4].

It is nevertheless conceivable that the ouabain-induced increase in ^{86}Rb outflow is also attributable, to a limited extent, to the activation of voltage-sensitive K^+ channels [7].

In the case of glucose, the activation of the Ca^{2+}-sensitive K^+ permeability is thought to be the consequence of stimulated Ca^{2+} inflow [9, 23, 24]. In the case of ouabain, however, the activation of the Ca^{2+}-sensitive K^+ permeability apparently resulted both from a stimulation of Ca^{2+} inflow and from the mobilization of Ca^{2+} from intracellular storing sites. That ouabain activated K^+ permeability, in part, by stimulating Ca^{2+} inflow is suggested by the observation that the increase in ^{86}Rb outflow evoked by ouabain was partially reduced in the absence of extracellular Ca^{2+} and attended by a dramatic increase in ^{45}Ca efflux, which itself was completely abolished in the absence of extracellular Ca^{2+}. The stimulation, by various secretagogues, of Ca^{2+} inflow into the B-cell is known to induce an increase in ^{45}Ca efflux, which is usually suppressed in islets exposed to Ca^{2+}-deprived media [17]. Ouabain may increase Ca^{2+} inflow into the B-cell by depolarizing the plasma membrane and hence gating voltage-sensitive Ca^{2+} channels [34]. On the other hand, the view that ouabain may, in addition, displace intracellular Ca^{2+} is suggested by the finding that ouabain still increased ^{86}Rb outflow in the absence of extracellular Ca^{2+}, although to a lesser extent than in the presence of extracellular Ca^{2+}. By inhibiting the Na^+,K^+-ATPase, ouabain may increase the intracellular concentration of Na^+ and hence displace Ca^{2+} from intracellular organelles by a process of Na/Ca exchange [16]. The existence of such a process at the level of intracellular organelles has been previously postulated in the islets [11, 16].

The increase in ^{86}Rb outflow induced by ouabain in glucose-deprived islets (Fig. 1) probably resulted for its major part from such an intracellular displacement, since under the latter experimental condition, ouabain, although depolarizing the B-cell fails to elicit electrical activity with concomitant gating of Ca^{2+} channels [14, 34]. In the absence of glucose, the increase in both ^{86}Rb and ^{45}Ca outflow was a progressive phenomenon and failed to display the biphasic pattern characteristically found in the presence of glucose and at normal Ca^{2+} concentration. The gradual increase seen in the absence of glucose is reminiscent of the gradual increase in ^{86}Rb outflow induced by ouabain in islets perifused in the presence of 8.3 mM glucose but absence of extracellular Ca^{2+}. Under the latter condition, ouabain is also unable to facilitate ^{40}Ca inflow and to enhance ^{45}Ca outflow. Incidentally, the failure of ouabain to augment ^{45}Ca outflow in the islets exposed to glucose but deprived of extracellular Ca^{2+} does not necessarily detract from the view that ouabain provokes an intracellular redistribution of Ca^{2+}. First, it should be kept in mind that glucose inhibits Ca^{2+} outflow at the level of the plasma membrane [18] and may mask, therefore, the increase in ^{45}Ca outflow otherwise expected from the intracellular redistribution of Ca^{2+}. For instance, glucose inhibits the outflow of ^{45}Ca from islets exposed to K^+-deprived media [16]; in such islets, the Na^+,K^+-ATPase is also inhibited [16]. Second, the failure of ouabain to increase ^{45}Ca efflux, from islets perifused in the presence of 8.3 mM glucose but absence of extracellular Ca^{2+} may result in addition from a relative depletion of intracellular Ca^{2+} stores, a phenomenon known to occur in islets exposed for a prolonged period to Ca^{2+}-deprived media [32].

The Effect of Glucose upon the Na^+,K^+-ATPase

In further experiments, we have examined whether a rise in the glucose concentration from an intermediate to a high value may activate the Ca^{2+}-sensitive modality of K^+ transport as a result of

a primary inhibitory action on the Na^+,K^+-ATPase.

The finding that a rise in the glucose concentration from an intermediate to a high value failed to stimulate ^{86}Rb outflow from islets perifused in the presence of ouabain is in agreement with such a possibility. Incidentally, in the presence of ouabain, the rise in glucose concentration apparently failed to affect Ca^{2+} inflow, but caused a modest fall in ^{86}Rb outflow. This phenomenon is reminiscent of that observed in the absence of extracellular Ca^{2+} in response to the same rise in glucose concentration [9, 28]. This suggests that the rise in glucose concentration causes a modest decrease in K^+ permeability, whenever the effects of such a rise on Ca^{2+} influx are minimized.

The view that glucose may inhibit the Na^+,K^+-ATPase appears incompatible, however, with the finding that glucose failed to decrease ^{86}Rb influx. The latter observation, which is in good agreement with prior reports [26, 35] contrasts with the dramatic reduction in ^{86}Rb inflow evoked by ouabain, whether in the presence of 8.3 or 16.7 mM glucose. These contrasting situations strongly suggest that glucose does not inhibit the islet Na^+,K^+-ATPase and, hence, that the increase in ^{86}Rb outflow and the enhancement of electrical activity seen when the glucose concentration is raised from an intermediate to a high value does not result from such an inhibition. With the exception of an isolated report [25], both biochemical [20] and bioelectrical data [14] also suggest that glucose does not exert any direct effect on the sodium pump activity in pancreatic B-cells.

It could be argued that the increase in ^{86}Rb outflow induced by ouabain was of much larger magnitude than that induced by the rise in the glucose concentration. Hence, the hypothetical effect of glucose to inhibit the Na^+,K^+-ATPase could be of such a modest magnitude as to prevent its detection by the measurement of ^{86}Rb influx. An inhibitory effect of glucose on Na^+,K^+-ATPase appears unlikely, however, since the effect of ouabain to stimulate ^{86}Rb outflow was not less pronounced in the presence of 16.7 mM than 8.3 mM glucose. If glucose, in high concentration, were to inhibit the Na^+,K^+-ATPase, one would expect the stimulatory effect of ouabain upon ^{86}Rb outflow to be less pronounced in the presence of a high rather than an intermediate concentration of glucose. Incidentally, the failure of glucose in high concentration to suppress the ouabain-induced increase in ^{86}Rb outflow further supports the view that glucose does not inhibit the response to cytosolic Ca^{2+} of the Ca^{2+}-sensitive modality of K^+ extrusion [4, 9, 23, 24].

In conclusion, the present data indicate that inhibition of the Na^+,K^+-ATPase by ouabain reproduces the effect of a rise in glucose concentration from an intermediate to a high value in stimulating the efflux of ^{86}Rb from perifused islets. However, inhibition of the Na^+,K^+-ATPase does not seem to represent the mechanism by which glucose in high concentration produces such a stimulation in ^{86}Rb outflow. Our data are also in agreement with recent electrophysiological studies which do not suggest that cyclic variations in the Na^+,K^+-ATPase activity play any significant role in the determinism of the burst pattern for electrical activity [2, 33, 38]. Hence, the mechanism by which high concentrations of glucose change the burst pattern of electrical activity into a continuous firing remains to be elucidated.

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References

1. Armando-Hardy, M., Ellory, J.C., Ferreira, H.G., Fleminger, S., Lew, V.L. 1975. Inhibition of the calcium-induced increase in the potassium permeability of human red blood cells by quinine. *J. Physiol. (London)* **250**:32P–33P
2. Atwater, I. 1980. Control mechanisms for glucose-induced changes in the membrane potential of mouse pancreatic B-cell. *Cien. Biol.* **5**:299–314
3. Atwater, I., Beigelman, P.M. 1976. Dynamic characteristics of electrical activity in pancreatic B-cells. I. Effects of calcium and magnesium removal. *J. Physiol. (Paris)* **72**:769–786
4. Atwater, I., Dawson, C.M., Ribalet, B., Rojas, E. 1979. Potassium permeability activated by extracellular calcium ion concentration in the pancreatic B-cell. *J. Physiol. (London)* **288**:575–588
5. Atwater, I., Meissner, H.P. 1975. Electrogenic sodium pump in B-cells of islets of Langerhans. *J. Physiol. (London)* **247**:56P–58P
6. Atwater, I., Ribalet, B., Rojas, E. 1978. Cyclic changes in potential and resistance of the B-cell membrane induced by glucose in islet of Langerhans from mouse. *J. Physiol. (London)* **278**:117–139
7. Atwater, I., Ribalet, B., Rojas, E. 1979. Mouse pancreatic B-cells: Tetraethylammonium blockade of the potassium permeability increase induced by depolarization. *J. Physiol. (London)* **288**:561–574
8. Boschero, A.C., Kawazu, S., Duncan, G., Malaisse, W.J. 1977. Effect of glucose on K^+ handling by pancreatic islets. *FEBS Lett.* **83**:151–154
9. Carpinelli, A.R., Malaisse, W.J. 1981. Regulation of ^{86}Rb outflow from pancreatic islets: The dual effect of nutrient secretagogues. *J. Physiol. (London)* **315**:143–156
10. Dean, P.M., Matthews, E.K. 1970. Glucose-induced electrical activity in pancreatic islet cells. *J. Physiol. (London)* **210**:255–264
11. Donatsch, P., Lowe, D.A., Richardson, B.P., Taylor, P. 1977. The functional significance of sodium channels in pan-

creatic beta-cell membranes. *J. Physiol. (London)* **267**:357–376
12. Henquin, J.C. 1978. D-glucose inhibits potassium efflux from pancreatic islet cells. *Nature (London)* **271**:271–273
13. Henquin, J.C. 1979. Opposite effects of intracellular Ca^{2+} and glucose on K^+ permeability of pancreatic islet cells. *Nature (London)* **280**:66–68
14. Henquin, J.C., Meissner, H.P. 1982. The electrogenic sodium-potassium pump of mouse pancreatic B-cell. *J. Physiol. (London)* **332**:529–552
15. Henquin, J.C., Meissner, H.P., Preissler, M. 1979. 9-aminoacridine and tetraethylammonium-induced reduction of the potassium permeability in pancreatic B-cells. Effect on insulin release and electrical properties. *Biochim. Biophys. Acta* **587**:579–592
16. Herchuelz, A., Malaisse, W.J. 1979. Regulation of calcium fluxes in pancreatic islets: Dissimilar effects of glucose and of sodium ion accumulation. *J. Physiol. (London)* **302**:263–280
17. Herchuelz, A., Malaisse, W.J. 1982. Calcium and insulin release. *In:* The Role of Calcium in Biological Systems. L.J. Angileri, editor. Vol. III, part II, p. CRC Press, Boca Raton, Florida (*in press*)
18. Herchuelz, A., Sener, A., Malaisse, W.J. 1980. Regulation of calcium fluxes in rat pancreatic islets: Calcium extrusion by sodium-calcium countertransport. *J. Membrane Biol.* **57**:1–12
19. Herchuelz, A., Thonnart, N., Carpinelli, A.R., Sener, A., Malaisse, W.J. 1980. Regulation of calcium fluxes in rat pancreatic islets. The role of K^+ conductance. *J. Pharmacol. Exp. Ther.* **215**:213–220
20. Kemmler, W., Löffler, G. 1977. NaK-ATPase in rat pancreatic islets. *Diabetologia* **13**:235–238
21. Lacy, P.E., Kostianovsky, M. 1967. Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* **16**:35–39
22. Lebrun, P., Malaisse, W.J., Herchuelz, A. 1981. Effect of calcium antagonists on potassium conductance in islet cells. *Biochem. Pharmacol.* **30**:3291–3294
23. Lebrun, P., Malaisse, W.J., Herchuelz, A. 1982. Paradoxical activation by glucose of quinine-sensitive potassium channels in the pancreatic B-cell. *Biochem. Biophys. Res. Commun.* **107**:350–356
24. Lebrun, P., Malaisse, W.J., Herchuelz, A. 1982. Activation but not inhibition by glucose of Ca^{2+}-dependent K^+ permeability in the rat pancreatic B-cell. (*Submitted to Biochem. Biophys. Acta*)
25. Levin, S.R., Kasson, B.G., Driessen, J.F. 1978. Adenosine triphosphatase of rat pancreatic islets. Comparison with those of rat kidney. *J. Clin. Invest.* **62**:692–701
26. Malaisse, W.J., Boschero, A.C., Kawazu, S., Hutton, J.C. 1978. The stimulus-secretion coupling of glucose-induced insulin release. XXVII. Effect of glucose on K^+ fluxes in isolated islets. *Pfluegers Arch.* **373**:237–242
27. Malaisse, W.J., Herchuelz, A. 1982. Nutritional regulation of K^+ conductance: An unsettled aspect of pancreatic B-cell physiology. *In:* Biochemical actions of Hormones. IX. G. Litwack, editor. pp. 69–92. Academic Press, New York
28. Malaisse, W.J., Lebrun, P., Herchuelz, A. 1982. Ionic determinants of bioelectrical spiking activity in the pancreatic B-cell. *Pfluegers Arch.* **395**:201–203
29. Matthews, E.K., O'Connor, M.D.L. 1979. Dynamic oscillations in the membrane potential of pancreatic islet cells. *J. Exp. Biol.* **81**:75–91
30. Meissner, H.P. 1976. Electrical characteristics of the beta-cells in pancreatic islets. *J. Physiol. (Paris)* **72**:757–767
31. Meissner, H.P., Preissler, M., Henquin, J.C. 1979. Possible ionic mechanisms of the electrical activity induced by glucose and tolbutamide in pancreatic B cells. *In:* Diabetes. W.K. Waldhäusl, editor. No. 500. Elsevier-North Holland International Congress Series, Amsterdam
32. Ravazzola, M., Malaisse-Lagae, F., Amherdt, M., Perrelet, A., Malaisse, W.J., Orci, L. 1976. Patterns of calcium localization in pancreatic endocrine cells. *J. Cell Sci.* **27**:107–117
33. Ribalet, B., Beigelman, P.M., 1979. Cyclic variation of K^+ conductance in pancreatic B-cells: Ca^{2+} and voltage dependance. *Am. J. Physiol.* **237**:C137–C146
34. Ribalet, B., Beigelman, P.M. 1982. Effect of sodium on β-cell electrical activity. *Am. J. Physiol.* **242**:C296–C303
35. Sehlin, J., Täljedal, I.-B. 1974. Transport of rubidium and sodium in pancreatic islets. *J. Physiol. (London)* **242**:P505–P515
36. Sehlin, J., Täljedal, I.-B. 1975. Glucose-induced decrease in Rb^+ permeability in pancreatic B-cells. *Nature (London)* **253**:635–636
37. Sener, A., Kawazu, S., Malaisse, W.J. 1980. The stimulus-secretion coupling of glucose-induced insulin release. Metabolism of glucose in K^+ deprived islets. *Biochem. J.* **186**:183–190
38. Tarvin, J.T., Pace, C.S. 1981. Glucose induced electrical activity in the pancreatic β-cell: Effect of veratridine. *Am. J. Physiol.* **240**:C127–C134

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