EFFECT OF GLUCOSE ON K^+ HANDLING BY PANCREATIC ISLETS

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1. Introduction

The handling of $^{86}Rb^+$ by pancreatic islets was recently investigated [1,2] with the view of characterizing the movements of K^+ in islets cells. The fate of $^{86}Rb^+$, however, may not be identical to that of $^{39}K^+$. The present study was undertaken to assess, by both radioisotopic procedure and direct measurement of $^{39}K^+$, the effect of glucose upon K^+ handling in isolated islets of Langerhans.

2. Materials and methods

2.1. *Metabolism of ^{42}K in the islets*

All experiments were performed with isolated islets removed from fed female albino rats [3]. For measurement of $^{42}K^+$ net uptake, groups of 10 islets each placed in polythene microcentrifuge tubes were first preincubated for 30 min at 37°C in 0.05 ml non-radioactive medium [3] containing sucrose (1 mM) and, when required, glucose (16.7 mM). A second aliquot of medium enriched with ^{42}K (28 μCi/ml; IRE, Fleurus, Belgium) and [6,6′(*n*)-^{3}H] sucrose (50 μCi/ml; Radiochemical Centre, Amersham, England) was then added to the tubes, which were incubated for 90–120 min. Thereafter, a first centrifugation (5 s; Beckman Microfuge, Model 152) was performed to deposit the islets in the tip of the tube. Di-*n*-butyl phthalate (0.1 ml; BDH Chemicals, Poole, England) was then layered on the top of the incubation medium, and a second centrifugation (10 s) performed to separate the islets pellet from the medium. The lowest two mm of the tube were removed with a scalpel, transferred to a scintillation vial containing a solution of EGTA (1.0 ml, 2 mM, pH 7.0), mixed with 10 ml of scintillation fluid (Instagel; Packard, Downer Groves, IL), and examined for their ^{42}K and ^3H content.

For measurement of ^{42}K efflux, groups of 140–180 islets each were preincubated for 60 min in a medium (1.0 ml) containing glucose (2.8 mM) and $^{42}K^+$ (28 μCi/ml), washed twice, and eventually placed in a perifusion chamber [4]. The perifusate was delivered at a rate of 1 ml/min. The effluent medium was collected from 6–60 min of perifusion over successive periods of 1 min each, and then examined for its radioactive content.

In all experiments, the extracellular K^+ concentration was maintained at 5 meq/litre. All measurements of ^{42}K were performed by liquid scintillation and corrected for radioisotopic decay.

2.2. *Measurement of ^{39}K in the islets*

After 90 min incubation, groups of 2000 islets each (mean protein content: 0.54 μg/islet) were transferred to polythene tubes and washed 3 times with 1.0 ml Tris buffer (150 mM, buffered to pH 7.3 with HCl) itself prepared with bidistilled water and stored in a polythene container. The washing procedure [3] was performed at room temperature and took 15–20 min to complete. Samples (1.0 ml) of the Tris buffer, as well as the two last washing media, were also placed in polythene tubes. After lyophilization, each sample was successively homogenized in 2 ml trichloroacetic acid (TCA) (5%) and centrifuged, an aliquot of the supernatant solution being then mixed with H_2O and assayed for its content in Mg and K by atomic absorption. The concentration of Mg (14 ± 4 μM) and K (41 ± 11 μM) in the last washing media, although

being somewhat higher than that of the Tris buffer (Mg, 7 ± 2 μM; K, 7 ± 1 μM), was usually low enough not to represent a significant source of contamination. Indeed, the amount of Mg and K present in the small volume (less than 0.1 ml) of washing medium possibly left on the islets represented no more than 2% of the total amount of Mg or K measured in the islets sample. In 2 out of 8 samples, however, a significant contamination of the islets preparation by residual extracellular cations was suspected on the basis, inter alia, of an abnormally high value for both K and Mg in the islets sample (see footnote in the Results section).

All results are expressed as the mean ± SEM.

3. Results and discussion

The amount of $^{42}K^+$ present in the islets, in excess of that attributable to contamination by extracellular fluid, was not significantly different at 90 min and 120 min incubation, respectively (table 1). This finding suggests that isotopic equilibration between extra- and intracellular K^+ was reached, so that the net uptake of $^{42}K^+$ could be used to calculate the size of the K^+ pool in the islets cells under steady-state conditions. Glucose (16.7 mM) increased the size of the K^+ pool from 161 ± 11 to 207 ± 13 pmol/islet ($P < 0.01$). This finding confirms data reported several years ago [5]. Ouabain (0.1 mM) markedly decreased the net uptake of ^{42}K ($P < 0.05$ or less), whether in the presence or absence of glucose, but apparently failed to suppress the proper effect of glucose.

The data obtained by the isotopic procedure were in fair agreement with the measurement of $^{39}K^+$ in islets incubated for 90 min in the absence or presence of glucose (16.7 mM). Whereas glucose failed to exert any obvious effect on the Mg^{2+} content of the islets (50 ± 9 pmol/islet; $n = 8$), it increased their K^+ content from a basal value of 172 ± 53 to 300 ± 60 pmol/islet ($n = 4$ in each case)*. The effect of glucose to increase the K^+ pool was significant when judged from the K^+/Mg^{2+} ratio (pmol/pmol), which averaged 3.87 ± 0.27 and 5.63 ± 0.47 ($n = 4$ in each case; $P < 0.02$) in the control and glucose-stimulated islets, respectively.

Further experiments were designed to decide whether the glucose-induced accumulation of K^+ was due to an increase in the influx and/or a decrease in the efflux of K^+ across the islets cells plasma membrane. In perifused islets prelabelled with $^{42}K^+$, the effluent radioactivity decreased in an exponential fashion (see insets of fig.1), with a mean slope of 8.88 ± 0.34 and 4.23 ± 0.74 percent/min ($P < 0.005$) in the absence and presence of glucose (16.7 mM), respectively. Taking into account these slopes and the absolute values for $^{42}K^+$ efflux (see legend to fig.1), it was possible to compute the integrated efflux of $^{42}K^+$ over an infinite period starting at time zero of the perifusion. Such an integrated value for $^{42}K^+$ efflux, which should be representative of the amount of $^{42}K^+$ present in the islets at the onset of perifusion, was not significantly different in the two series of experiments ($P > 0.3$) and, for the group as a whole, averaged 130 ± 17 pmol K^+. The latter value is somewhat lower than that found for the net uptake of $^{42}K^+$ (table 1), as expected from both the length of preincubation with $^{42}K^+$ (60 min) and the washing procedure used whilst transferring the islets from the preincubation medium to the perifusion chamber.

The glucose-induced difference in slope of the regression lines characterizing the rate of fall in effluent $^{42}K^+$ indicates that the sugar decreases the fractional outflow rate of K^+, possibly by reducing the permeability of the plasma membrane to effluent K^+. The effect of glucose on K^+ efflux was an almost immediate phenomenon. Thus, within 2 min after the addition of glucose (16.7 mM) to the perifusate, there was an abrupt fall in effluent $^{42}K^+$ (fig.1). Because of the latter phenomenon, the values for $^{42}K^+$ efflux recorded 3–5 min after the addition of glucose were significantly lower ($P < 0.001$) than those expected from the extrapolation of the regression line computed over the 5 min period immediately preceding the administration of glucose. The effect of glucose on K^+ efflux was also rapidly reversible.

Assuming even distribution of K^+ in a single cellular pool, the inflow–outflow rate of K^+, under steady-state conditions, can be calculated as the product of the size of the K^+ pool (as judged from the values for

* Excluding from each group of measurements one abnormally high value (i.e., superior to the mean ± 3 SD), the K^+ concentration averaged 121 ± 19 and 246 ± 39 pmol/islet ($n = 3$ in each case; $P < 0.05$) after incubation in the absence or presence of glucose respectively. The mean Mg^{2+} content of these six groups of 2000 islets each averaged 37 ± 5 pmol/islet

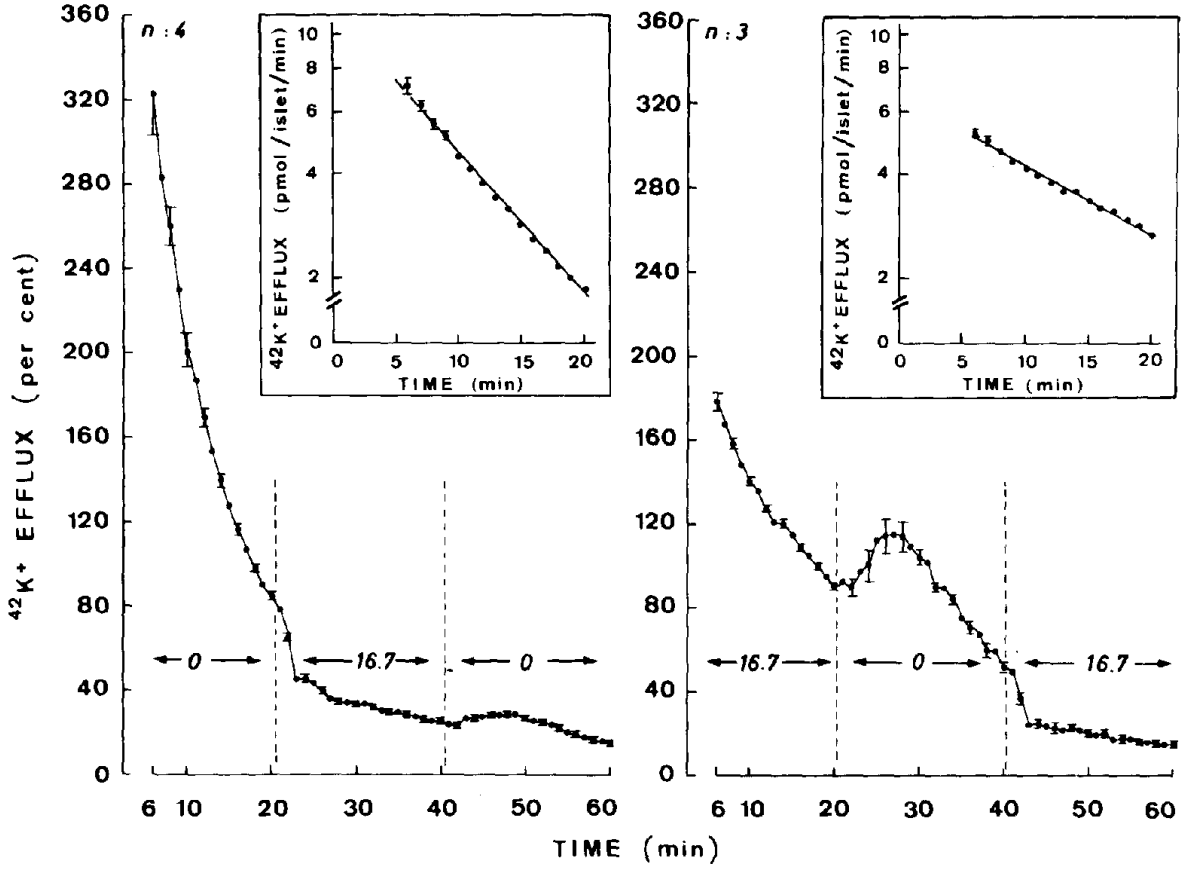

Fig.1. Effect of glucose upon $^{42}K^+$ efflux from perifused islets. At the time shown by the vertical dotted lines, glucose (16.7 mM) was either added or removed from the perifusate. The efflux of $^{42}K^+$ is shown relative to the mean value recorded within each experiment between 16 min and 20 min. When expressed as K^+ with the same specific activity as that of the preincubation medium, such a reference value averages 2.20 ± 0.22 (left panel) and 2.94 ± 0.18 (right panel) pmol/islet/min. In the insets, the data collected up to 20 min are illustrated in semilogarithmic coordinates. Also shown is the number of individual experiments (*n*).

Table 1

Glucose	Ouabain	90 min	120 min
–	–	100.0 ± 7.1 (24)	102.7 ± 10.1 (8)
–	0.1 mM	36.5 ± 10.2 (7)	
16.7 mM	–	124.5 ± 8.2 (24)	132.7 ± 6.6 (8)
16.7 mM	0.1 mM	57.2 ± 13.8 (7)	

Mean values (± SEM) for the net uptake of $^{42}K^+$ by islets incubated for 90 min or 120 min are expressed as percent of the mean basal value found within the same experiments at 90 min incubation; such a control value averaged 164 ± 13 pmol K^+/islet (n = 24). Also shown is the number of individual determinations (in parentheses)

$^{42}K^+$ net uptake shown in table 1) by its fractional turnover rate (as judged from the values for $^{42}K^+$ efflux illustrated in the insets to fig.1). In the absence of glucose, the inflow–outflow rate averaged 14.2 ± 1.6 pmol K^+/islet/min. The latter value which is identical to that measured with $^{86}Rb^+$ (14.8 ± 1.4 pmol K^+/islet/min) was not significantly different ($P > 0.09$) from the somewhat lower mean value found in the presence of glucose (16.7 mM). Incidentally, although the effect of glucose to increase the K^+ pool size, relative to basal value, appeared somewhat variable according to the method used ($^{42}K^+ \leqslant {}^{39}K^+ \leqslant {}^{86}Rb^+$), even the two-fold increase in K^+ pool suggested by some of these measurements was not sufficient to result in an increase of the calculated mean inflow–outflow rate. In other words, there was no indication that glucose increases the inflow rate of K^+ into the islets, a process likely to be mediated, in part at least, by an energy-requiring and ouabain-sensitive pumping process.

In summary, the present data confirm and extend the conclusions derived from previous experiments carried out with $^{86}Rb^+$ [1,2]. They suggest that glucose, by reducing the permeability of the plasma membrane to effluent K^+, increases the concentration of K^+ in the islets cells and under steady-state conditions, reduces proportionally the fractional turnover rate of the cellular K^+ pool. Glucose, however, does not increase the inflow–outflow rate of K^+ into the islets cells. The mechanism by which glucose affects the permeability of the plasma membrane to effluent K^+ remains to be elucidated.

Acknowledgements

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References

[1] Sehlin, J. and Täljedal, I.-B. (1974) J. Physiol. 242, 505–515.
[2] Boschero, A. C., Kawazu, S. and Malaisse, W. J. (1977) Diabetologia 13, in press.
[3] Malaisse-Lagae, F. and Malaisse, W. J. (1971) Endocrinology 88, 72–80.
[4] Malaisse, W. J., Brisson, G. R. and Baird, L. E. (1973) Amer. J. Physiol. 224, 389–394.
[5] Howell, S. L. and Taylor, K. W. (1968) Biochem. J. 108, 17–24.