Glucose reduces both Rb^+ influx and efflux in pancreatic islet cells

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Microdissected, β-cell-rich pancreatic islets from *ob/ob* mice were used in studies of $^{86}Rb^+$ transport. D-Glucose (20 mM) induced a biphasic reduction in $^{86}Rb^+$ efflux. The reduction stabilized within 10 min at 34% of the efflux rate at zero glucose. The initial $^{86}Rb^+$ uptake (5 min) was dose-dependently reduced by ouabain with maximum inhibition at 1 mM. D-Glucose (20 mM) did not affect the ouabain-sensitive $^{86}Rb^+$ influx but markedly reduced (48%) the ouabain-resistant isotope influx. The results suggest that D-glucose does not affect the Na^+/K^+ pump in pancreatic β-cells and that the glucose-sensitive K^+-transporting modalities (K^+ channels) in the β-cells can mediate both inward and outward K^+ flux.

(Pancreatic β-cell) Rb^+ flux K^+ flux Ouabain Na^+/K^+ pump D-Glucose

1. INTRODUCTION

K^+ is transported across the pancreatic β-cell membrane by a pump-leak system of a rather classical appearance [1,2]. Previous studies have shown that the efflux of Rb^+ or K^+, representing the K^+ permeability, is reduced by elevated glucose concentrations [3–5]. This effect is partly responsible for the glucose-induced electrical depolarizations of the β-cell membrane [3–6]. From studies of Na/K-activated ATPases in islet homogenates it has been proposed that part of the glucose actions in the β-cells could involve inhibition of the Na/K pump [7]. Previous measurements of islet K^+ uptake with $^{86}Rb^+$ [1,5] or $^{42}K^+$ [8] or β-cell membrane potential with microelectrodes [2] have not provided evidence for a direct effect of glucose on the Na/K pump. However, the measurements of K^+ or Rb^+ transport have not included attempts to separate ouabain-sensitive transport (Na/K pump) from ouabain-resistant flux. The present study was undertaken to establish in more detail whether glucose affects the $^{86}Rb^+$ influx into pancreatic β-cells and whether such an effect is restricted to the ouabain-sensitive or ouabain-resistant fractions of the $^{86}Rb^+$ influx. $^{86}Rb^+$ was used as a K^+ analogue [1,5] and parallel measurements of glucose effects on $^{86}Rb^+$ influx and efflux were performed to establish quantitative as well as qualitative comparisons of fluxes in both directions.

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2. MATERIALS AND METHODS

Adult, non-inbred *ob/ob* mice of a local colony (Umeå *ob/ob* mice) were used throughout. Prior to experiments, the animals were starved overnight to normalize the blood sugar levels. These mice are characterized by β-cell hyperplasia resulting in large islets with more than 90% β-cells [9]. The present results are therefore probably representative of this endocrine cell type. Although the animals are metabolically abnormal, their β-cells respond normally to various stimulators and inhibitors of insulin secretion [10].

Pancreatic islets were microdissected free-hand [11]. Then, batches of 4–5 islets (influx ex-

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periments) or 10–15 islets (efflux experiments) were preincubated for 30 min at 37°C in a modified Krebs-Ringer medium (KRH) with the following composition (in mM): 130 NaCl, 4.7 KCl, 2.56 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 20 Hepes. The gas phase was ambient air, the pH 7.4, and the preincubation medium contained 3 mM D-glucose. After preincubation, islets were incubated in the same type of basal medium for various time periods in short-term incubations (influx experiments) or 120 min (efflux experiments) essentially according to [1]. The medium was supplemented with 28 μM $^{86}Rb^+$ and 8 μM [6,6′-^{3}H]sucrose as extracellular marker. The glucose concentration during incubation with isotopes is indicated in the legend to fig.1. In the efflux experiments, the radiolabelled islets were perifused as described [12]. After incubation or perifusion, the islets were freeze-dried (−40°C, 0.1 Pa), weighed and their radioactive contents

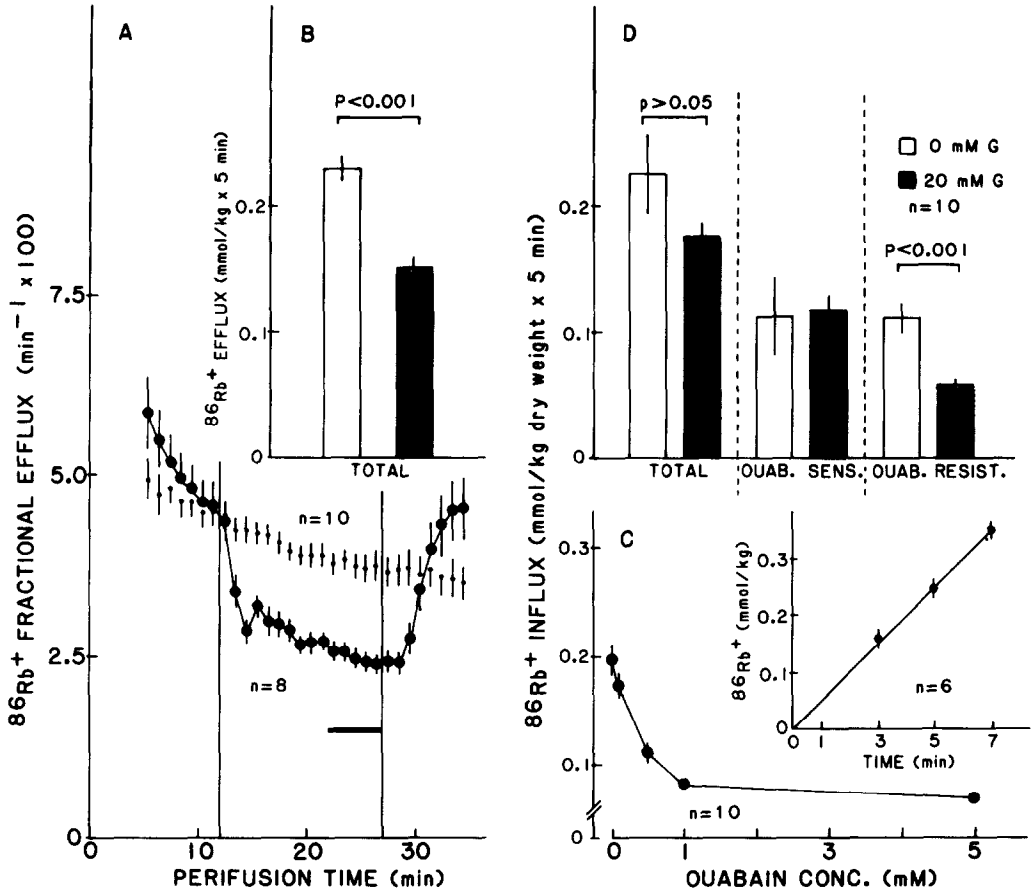

Fig.1. Effects of 20 mM D-glucose on $^{86}Rb^+$ influx and efflux. Microdissected pancreatic islets were preincubated for 30 min in KRH medium containing 3 mM D-glucose. In efflux experiments (A,B), the islets were labelled for 120 min in KRH medium containing 3 mM D-glucose and 28 μM $^{86}Rb^+$. They were then perifused with KRH medium containing either no D-glucose throughout (small filled circles in panel A and open bar in panel B) or initially zero D-glucose (min 0–12) followed by 20 mM D-glucose (min 13–27) and then back to zero D-glucose (min 28–35) (large filled circles in panel A and filled bar in panel B). Data in panel B have been calculated from the pooled efflux during min 23–27 in panel A. In uptake experiments (C,D), the islets were incubated with 28 μM $^{86}Rb^+$ and 8 μM [6,6′-^{3}H]sucrose for 5 min in the absence of D-glucose (C) or absence (open bars in D) or presence of 20 mM D-glucose (filled bars in D). Data represent mean values ± SE for the number of experiments indicated in the figure. Statistical significance was assessed by using the 2-tailed Student's *t*-test.

measured in a liquid scintillation spectrometer. The fractional efflux was calculated by dividing the content of $^{86}Rb^+$ in each 1 min fraction by the total islet content of $^{86}Rb^+$ at the beginning of that minute.

3. RESULTS AND DISCUSSION

Fig.1A shows the effects of 20 mM D-glucose on the $^{86}Rb^+$ efflux from prelabelled islets. A rapid reduction in the $^{86}Rb^+$ fractional efflux (cf. [3–5]) was followed by a transient increase (cf. [13]), in turn followed by a period of more stable but reduced efflux rate. When D-glucose was removed, the reduction was promptly and completely reversed. Fig.1B shows the $^{86}Rb^+$ efflux calculated from the mean fractional efflux during the last 5 min in 20 mM D-glucose or the corresponding time period in the control experiments (horizontal filled bar) and taking the total β-cell $^{86}Rb^+$ pool to be 1.21 ± 0.13 mmol/kg dry wt islets (mean ± SE of 6 separate experiments). D-Glucose reduced the $^{86}Rb^+$ efflux rate by 34% ($P < 0.001$).

Fig.1C shows experiments to characterize the experimental system for measuring $^{86}Rb^+$ influx in *ob/ob* mouse islets. The inset in fig.1C shows that incubation with $^{86}Rb^+$ for 3, 5 or 7 min resulted in linear time dependence of the uptake, suggesting that 5-min incubations represent unidirectional influx of $^{86}Rb^+$. This incubation time was then selected in further experimentation. The addition of ouabain to the incubation medium inhibited the $^{86}Rb^+$ influx in a dose-dependent manner. Maximum effect was obtained with 1 mM ouabain, which was selected as the dose for further experiments.

Fig.1D shows the effects of 20 mM D-glucose on $^{86}Rb^+$ influx. The 'total ^{86}Rb influx' in the absence of ouabain was slightly reduced by D-glucose (22%), although this effect was not highly significant statistically ($0.05 < P < 0.1$). This lack of significant glucose effect on total $^{86}Rb^+$ uptake is consistent with previous observations [1,5]. When the ouabain-sensitive component of the $^{86}Rb^+$ influx was extracted by computing, in each experiment, the difference between total ^{86}Rb influx and 'ouabain-resistant $^{86}Rb^+$ influx' (residual influx at 1 mM ouabain), it became obvious that the 'ouabain-sensitive $^{86}Rb^+$ influx' was not affected by 20 mM glucose (fig.1D). On the other hand, the ouabain-resistant influx was markedly reduced (48%).

Our results lead to two basic conclusions. First, it appears that 20 mM D-glucose does not affect the Na/K pump in intact β-cells. Second, both ouabain-resistant influx and efflux of $^{86}Rb^+$ (K^+ analogue) are inhibited by D-glucose, which may suggest that the glucose-sensitive units for passive K^+ transport (K^+ channels) can mediate K^+ flux in both directions. Similar results have been obtained with rat islets after preincubation with D-glucose [14]. The fact that the present two experimental systems for measurements of Rb^+ efflux and influx respectively give the same total flux rate in the absence of glucose (fig.1B,D) may allow quantitative comparisons of the glucose effects. It appears that the glucose-induced reduction of total efflux is larger than the reduction of total influx (34 vs 22%). This asymmetry may help to explain how prolonged incubations in the presence of high glucose concentrations lead to a gradual increase in the apparent equilibrium content of $^{86}Rb^+$ [5]. Future studies are needed to establish precisely how the glucose-sensitive but ouabain-resistant $^{86}Rb^+$ (K^+) influx is related to the glucose-sensitive $^{86}Rb^+$ efflux.

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