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Stimulation by D-Glucose of $^{36}Cl^-$ Efflux from Prelabeled Rat Pancreatic Islets

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D-glucose was previously reported to cause a concentration-related decrease in the $^{36}Cl^-$ content of prelabeled islets prepared from *ob/ob* mice, a current animal model of inherited obesity. From these findings, it was inferred that the hexose stimulates Cl^- efflux from islet cells and that such an increase in Cl^- permeability may partly mediate glucose-induced depolarization of insulin-producing cells. The aim of the present study was to investigate the possible extension of these findings to islets prepared from normal rats by measuring the changes evoked by increasing concentrations of D-glucose in $^{36}Cl^-$ outflow itself from prelabeled isolated islets. After 60 min preincubation at 37°C in the presence of 3 m*M* D-glucose and $^{36}Cl^-$ (75 µCi/mL), the islets were incubated for 8–10 min at 37°C in the presence of increasing concentrations of the hexose (3–20 m*M*). The changes in $^{36}Cl^-$ outflow during incubation indicated that D-glucose, in excess of a threshold concentration close to 5 m*M*, indeed increases effluent radioactivity from the prelabeled islets. It is proposed, therefore, that a gating of volume-sensitive anion channels in glucose-stimulated insulin-producing islet cells participates in the depolarization of the plasma membrane recorded in the range of insulinotropic concentrations of the hexose.

Key Words: Pancreatic islets; $^{36}Cl^-$ outflow; D-glucose.

Introduction

A gating of volume-sensitive anion channels may participate to the depolarization of the plasma membrane caused by high concentrations of D-glucose in insulin-producing B-cells of the endocrine pancreas *(1–3)*. The efflux of taurine from prelabeled cells is currently used to assess changes in the activity of such channels, e.g., in insulin-producing cells *(4)*. In rat isolated pancreatic islets, however, the pleiotropic effects of D-glucose upon effluent radioactivity from islets prelabeled with [1,2-^{3}H]taurine were considered to be poorly informative *(5)*, probably because of the vastly different content in taurine of B and non-B islet cells *(6)*. As an alternative approach, the effect of increasing concentrations of D-glucose on the efflux of $^{36}Cl^-$ from prelabeled rat islets was investigated in the present study.

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Results

After 60 min preincubation at 37°C, the $^{36}Cl^-$ content of the islets and surrounding medium averaged, when expressed by reference to the specific radioactivity of the preincubation medium, 358.4 ± 37.7 pmol/islet ($n = 65$). Assuming an intracellular space close to 2.84 nL per islet *(7)*, this would correspond to an intracellular Cl^- concentration of 126 ± 13 m*M*, as compared to 128 m*M* in islets from *ob/ob* mice also incubated for 60 min at 3.0 m*M* D-glucose in a Krebs–Henseleit medium *(8)*.

Over 8–10 min incubation at 3 m*M* D-glucose, the efflux of $^{36}Cl^-$ was not significantly different from that recorded in the islets immediately separated from the incubation medium at the onset of the final incubation (Fig. 1). Thus, the difference between these two sets of values averaged no more than 0.7 ± 6.9 % (d.f. = 22; $p > 0.9$) of the mean $^{36}Cl^-$ efflux found, within the same experiment(s) in the presence of 20 m*M* D-glucose.

The $^{36}Cl^-$ efflux recorded at 3, 5, and 10 m*M* D-glucose averaged 54.6 ± 6.3, 69.4 ± 6.8 and 78.2 ± 8.4% (n = 11–13) of the corresponding mean value found within the same experiment(s) at 20 m*M* D-glucose (100.0 ± 10.5%; $n = 13$). It was thus significantly lower at both 3 m*M* ($p < 0.005$) and 5 m*M* ($p < 0.025$) D-glucose than at 20 m*M* D-glucose and at 3 m*M* D-glucose than at 10 m*M* D-glucose ($p < 0.02$). The mean value recorded at 5 m*M* D-glucose exceeded ($p < 0.06$) that found in islets immediately separated from the incubation medium at the onset of the final incubation period.

When corrected for the readings made in the latter islets and taking into account the radioactive content of the islets at the onset of the final incubation period, the efflux of $^{36}Cl^-$ at 20.0 m*M* D-glucose averaged 23.1 ± 2.2 pmol/min per islet, corresponding to a fractional outflow rate close to 0.187 ± 0.017 min^{-1}. The latter value is virtually identical to that found by Sehlin, at the same hexose concentration, in islets prepared from *ob/ob* mice, i.e., 0.184 min^{-1} *(8)*.

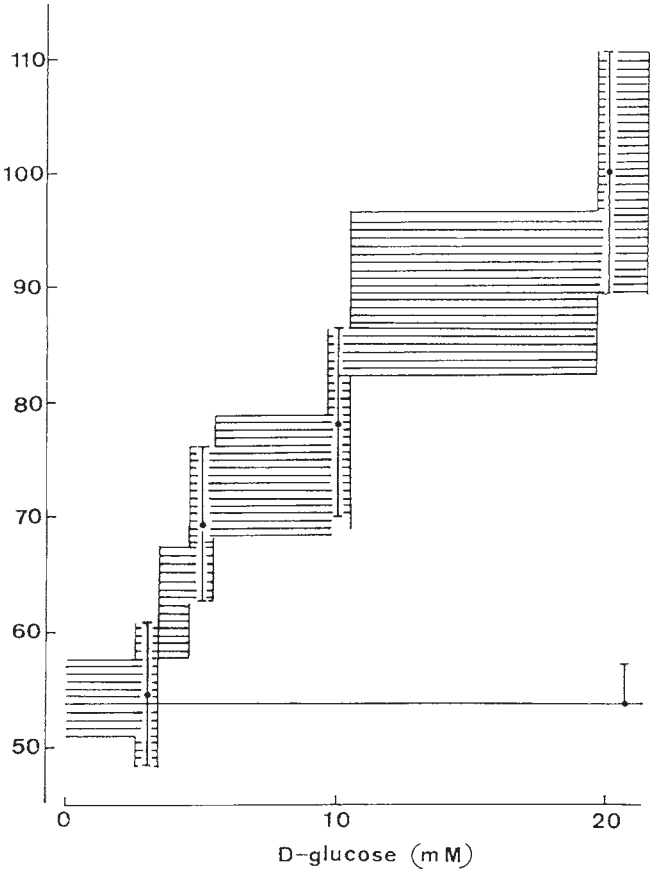

Fig. 1. Effect of increasing concentrations of D-glucose upon $^{36}Cl^-$ efflux from prelabeled islets. All results are expressed in percentage of the mean $^{36}Cl^-$ outflow recorded within the same experiment(s) at 20 m*M* D-glucose. The horizontal line at the bottom of the figure refers to islets immediately separated from the incubation medium at the onset of the final 8–10 min incubation period. The mean results (±SEM) under each experimental condition (closed circles) refer to 11–13 individual observations. The data illustrated in between given experimental measurements refer to the mean (±SEM) of such measurements (n = 24–25).

Figure 1 illustrates the effect of increasing concentrations of D-glucose upon $^{36}Cl^-$ efflux from prelabeled islets. It documents that the enhancing action of the hexose upon $^{36}Cl^-$ outflow displays a threshold value close to 5.0 m*M* (see above) and a half-maximal response close to 10.0 m*M*. These two features are similar to those characterizing the effect of D-glucose upon insulin release from rat islets *(9)*.

Discussion

The present results confirm, in a different species and by a different approach, the pilot information obtained by Sehlin in islets of *ob/ob* mice *(8)*. Thus, as already noted in the results section, the absolute values for both the Cl^- content of the islets and the rate constant for $^{36}Cl^-$ efflux were closely comparable in the prior and present investigations. Moreover, in both series of experiments, the threshold value for the enhancing action of D-glucose upon $^{36}Cl^-$ was close to 5 m*M*. The D-glucose concentration-response relationship, as documented in the present study, is also virtually identical to that obtained by Best when measuring the effect of increasing concentrations of D-glucose upon the channel open probability of a 200 ps anion-selective channel in recordings of cell-attached rat pancreatic B-cells *(3)*. Thus, in this case, the threshold value was again close to 5 m*M* D-glucose and a half maximal increment in channel open probability was recorded at a D-glucose concentration close to 10 m*M*.

The salient finding in these three series of investigations indeed consists in the fact that the concentration-related effect of D-glucose to cause the gating of the volume-sensitive anion channels closely parallels that of the hexose as an insulinotropic agent. This is in sharp contrast to the concentration response for the effect of D-glucose to provoke the closing of ATP-sensitive K^+ channels *(10)*. Thus, in the latter case, a maximal response is already recorded at a concentration of D-glucose close to 5 m*M*. In other words, it would appear that the major function of the ATP-sensitive K^+ channels is to hyperpolarize the B-cell, thus preventing release of insulin in situations of hypoglycemia. A somewhat comparable situation may prevail in neurons *(11)*. In the insulin-producing B-cell, the activation of the volume-sensitive anion channels by D-glucose is more likely to be involved in the regulation of electrical activity and insulin release in the hyperglycemic range of hexose concentrations between 5 and 20 m*M*.

Materials and Methods

Groups of about 800 islets each, prepared by the collagenase procedure *(12)* from fed female Wistar rats (Iffa Credo, L'Arbresle, France), were first preincubated for 30 min at 37°C in 0.45 mL of a Hepes-buffered (20 m*M*, pH 7.4) medium containing 140 m*M* NaCl, 5 m*M* KCl, 1 m*M* $MgCl_2$, 1 m*M* $CaCl_2$, 3 m*M* D-glucose, and 1.0 mg/mL bovine serum albumin. After removal of the medium, the islets were then preincubated for 60 min at 37°C in 0.45 mL of the same buffer now containing 75 μCi/mL $^{36}Cl^-$ (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). Working in a cold room (4°C), the radioactive preincubation medium was removed and the islets placed in a Petri dish in 4.0 mL of iced non-radioactive Hepes buffer. Groups of 30 islets each were then transferred to microfuge tubes (Tubo Beckman; Fullerton, CA, USA) containing 150–200 μL of the same iced Hepes buffer. After removal of such a medium and successive addition of 80 μL of the iced Hepes buffer and 150 μL of dibutyl phthalate (Sigma Chemical Co., St. Louis, MO, USA), some tubes were immediately centrifuged in the cold room for 3 min at 2000*g* in a Spectrafuge Mini (Labnet International, Inc., Woodbridge, NJ, USA), this corresponding to time zero of the final incubation. In the other tubes, after addition of 80 μL of the iced Hepes buffer

containing, as required, 3, 5, 10, or 20 m*M* D-glucose, the tubes were incubated for 8–10 min at 37°C and, after addition of 150 µL of dibutyl phthalate, centrifuged as indicated above. The islet pellet with some surrounding oil and an aliquot part (50 µL) of the final incubation medium were eventually examined for their radioactive content by liquid scintillation.

All results are presented as mean values (±SEM), together with the number of individual observations (*n*) or degree of freedom (d.f.). The statistical significance of differences between mean values was assessed by use of Student's *t*-test. The results of this statistical analysis were confirmed by analysis of variance and Bonferroni's multiple comparison test.

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