ESTIMATION OF POTASSIUM PERMEABILITY IN MOUSE ISLETS OF LANGERHANS

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ABSTRACT

Measurements of $^{86}\text{Rb}^+$ efflux from perfused collagenase-isolated mouse islets and membrane potential from microdissected mouse islets have been used to estimate, using the Goldman model, the potassium permeability coefficient (P_K) and potassium conductance (g_K) of the cell membrane. In the presence of low glucose (2.8 mM) $P_K = 2.5 \times 10^{-9} \text{ m sec}^{-1}$ and $g_K = 0.22 \text{ S m}^{-2}$ and at high glucose (22.2 mM) $P_K = 0.96 \times 10^{-9} \text{ m sec}^{-1}$ and $g_K = 0.13 \text{ S m}^{-2}$. There was a tendency for P_K to fall slowly with time. Following return to low glucose, there was a transient decrease in efflux rate constant. However, this is not reflected in the calculated P_K . It is clear that flux data for ions, in the absence of membrane potential measurements, should be interpreted with caution.

From studies on *ob/ob* mouse islets, Sehlin and Täljedal (15) proposed that a decrease in potassium permeability is responsible for the glucose-induced depolarization of pancreatic β -cells. Subsequently, other workers (3, 4, 10, 11, 14) confirmed, using $^{42}\text{K}^+$ and $^{86}\text{Rb}^+$, that an increase in glucose concentration reduced the ion flux in rat islets. It was shown that $^{86}\text{Rb}^+$ was a satisfactory tracer for potassium (4, 10). Measurements of input resistance in mouse islets showed that membrane conductance was decreased by high glucose (1). In order to determine the permeability coefficient and the conductance of the cell membrane from the efflux rate constant, it is necessary to know the membrane potential and this has not been determined in rat islets.

The data presented here, obtained using normal mouse islets, confirm quantitatively the inhibitory effect of high glucose on $^{86}\text{Rb}^+$ efflux found in rat islets. However, using values of the membrane potential in low and high glucose, the results for mouse islets can be expressed in terms of permeability coefficients and conductances, calculated using the Goldman model.

MATERIALS AND METHODS

The solution used was modified Krebs; 120 mM NaCl, 25 mM NaHCO_3 , 5 mM KCl, 2.56 mM CaCl_2 and 1.1 mM MgCl_2 equilibrated with 95% $\text{O}_2/5\% \text{ CO}_2$ at 37°C to maintain the pH at 7.4 and containing either 2.8 or 22.2 mM glucose. For flux experiments, the medium also contained 5 mg cm^{-3} bovine serum albumin (Fraction V Armour Pharmaceuticals, Eastbourne, England).

Isolated islets were prepared from normal albino mice using collagenase (Sigma, type V). 100–150 islets were incubated at 37°C for 2 h in 0.1 cm^3 of medium containing 2.8 mM glucose and $10 \mu\text{Ci}$ of $^{86}\text{Rb}^+$ (Amersham International, specific activity $0.3\text{--}0.5 \text{ mCi/mg}$). After washing three times at room temperature with 2.5 cm^3 of unlabelled medium, the islets were transferred to the perfusion chamber. This was a modified plastic syringe with volume adjusted to 0.2 cm^3 . The flow rate was $0.5 \text{ cm}^3 \text{ min}^{-1}$ and the half-time for the perfusion system was 27 sec. The perfusate was collected at 1 min intervals. $^{86}\text{Rb}^+$ in the samples and the residual remaining in the islets at the end of the experiment was counted using β -scintillation counting (Picofluor 15).

The efflux rate constant was computed as

$$k_{t+\Delta t} = -\frac{1}{\Delta t} \log_e \left(\frac{A_{t+\Delta t}}{A_t} \right),$$

where A_t is amount of $^{86}\text{Rb}^+$ in the islets at the beginning of the time interval and $A_{t+\Delta t}$ is the amount present at the end of the time interval Δt . The first 15 min of the efflux experiment was disregarded due to carry-over of extracellular $^{86}\text{Rb}^+$.

The membrane potential was measured in microdissected mouse islets as described previously (1). The membrane potential is given as the potential of the inside of the cell with respect to the medium.

RESULTS AND DISCUSSION

The mean value for the efflux rate constant is given as a function of time in Fig. 1A. The efflux rate constant decreased slowly with time from 0.027 min^{-1} at 15 min to 0.019 min^{-1} at 95 min. Raising glucose to 22.2 mM caused a rapid fall in the efflux rate constant followed by a steady decline from 0.019 min^{-1} to 0.016 min^{-1} during the 45 min exposure to high glucose. This decrease in rate constant suggests that the islets might contain a small slowly exchanging compartment. Henquin and Meissner (12) also noted a decrease in $^{86}\text{Rb}^+$ efflux in normal mouse islets when glucose was increased from 0 to 10 mM. More extensive data on rat islets indicated comparable changes in $^{86}\text{Rb}^+$ efflux (3, 4, 10–12, 14). However, the data in Fig. 1A show that the reduction in glucose from 22.2 to 2.8 mM resulted in a sharp transient decrease in efflux rate constant to 0.009 min^{-1} with return to the expected values within 10 min.

The β -cell membrane potentials of 10 microdissected mouse islets were measured in the presence of 2.8 and 22.2 mM glucose and are

Table 1 *Effect of Glucose on Membrane Parameters*

	Glucose concentration	
	2.8 mM	22.2 mM
E (mV)	-60 ± 2	-34 ± 2
k_{Rb} (min^{-1})	0.024	0.017
P_K (m sec^{-1})	2.5×10^{-9}	0.96×10^{-9}
g_K (S m^{-2})	0.22	0.13

The efflux rate constants are the mean value for 5 min before the glucose concentration was raised and the mid-point value in the high glucose medium. E is given as mean $\pm$ SEM.

given in Table 1. In 2.8 mM glucose no electrical activity was seen whereas continuous spike activity was observed in 22.2 mM glucose. The membrane potential in 22.2 mM glucose was measured at the base of the spikes. The change in membrane potential when raising or lowering glucose was complete within 2 min.

The permeability coefficient and membrane conductance are more closely related to the properties and functions of the cell membrane than the value for the efflux rate constant. In order to calculate the permeability coefficient (P_K) it was assumed that 1) Rb^+ behaved in exactly the same way as K^+ 2) the efflux is a passive diffusion process 3) the Rb^+ flux was unidirectional 4) the cell membrane and not the extracellular space was rate limiting. For a passively diffusing ion, the relation between the permeability coefficient (P_j) and efflux rate constant (k_j) involves the membrane potential. The relationship can be determined using the Goldman constant-field model (6, 9). Then

$$P_j = k_j \frac{V}{A} \left[\frac{1}{aE} (1 - \exp \{-aE\}) \right]$$

where k_j is the efflux rate constant, V/A is the volume/area ratio of a β -cell (strictly the volume should be the volume of water in the cell) with the β -cell being regarded as a sphere of radius $5 \mu\text{m}$, $a = z_j F/RT$ and E is the membrane potential. The term within the outer square brackets indicates the influence of the membrane potential on the ion flux. The calculated values of P_K as a function of time are given in Fig. 1B. The membrane potential was assumed to have reached the new value by the second minute after the changes in glucose concentration. The transient decrease in efflux rate constant (Fig. 1A) on return to low glucose might suggest a transient fall in potassium permeability. However, when the permeability coefficient is calculated using the membrane potential, no transient is seen. It should be stressed that measurements of ion flux not accompanied by measurements of membrane potential provide very restricted information about the properties of the cell membrane. It is clear that the change in potassium permeability is much larger than is apparent from the efflux data.

The relationship between membrane slope conductance (g_j) and P_j can be obtained by differentiation of the Goldman flux equation. Then

$$g_j = P_j \frac{az_j F}{(1 - \exp \{aE\})^2} [(C_o - C_i)aE \exp \{aE\} + (C_o - C_i \exp \{aE\})(1 - \exp \{aE\})],$$

Fig. 1 Effect of glucose on (A) efflux rate constant and (B) potassium permeability coefficient, P_K . The glucose concentration was raised to 22.2 mM at 30 min and returned to 2.8 mM at 75 min. The data are the mean of 4 experiments. Error bars represent SEM of the data normalised to 26–29 min as reference.

where C_o and C_i are the external and intracellular concentrations of ion j respectively. This equation makes no assumption that the intracellular ion is in equilibrium with the medium (the potassium ion cannot be in equilibrium at both membrane potentials). If the ion is in equilibrium, the second term within the square brackets disappears. The intracellular potassium concentration is taken as 110 mM. The values of g_K at low and high glucose are given in Table 1. As g_K is likely to be a major component of total membrane conductance, the data predict that the electrical conductance of the membrane would be low.

Direct electrical measurements of the input resistance of the β -cell give values of about $10^8 \Omega$ (1). This corresponds to a conductance of about 30 S m^{-2} , considerably higher than the values obtained from flux data (Table 1). However, such measurements are affected by the

extent of electrical coupling between the cells. Treating the system as a volume conductor, the membrane resistance in the presence of 11.1 mM glucose has been estimated as $0.64 \times 10^6 \Omega \text{ cm}^2$ (8). This is equivalent to a membrane conductance of 0.016 S m^{-2} , a value lower than that obtained from the flux data. However, it was pointed out that the quality of fit of the electrical data was rather insensitive to the value of membrane resistance and thus membrane resistance was not well defined (8). The membrane conductances estimated by electrical methods are affected by the complexity of intercellular coupling within the islet whereas flux measurements are independent of cell coupling.

It is of interest to compare the value of P_K for mouse β -cells with values that have been found in other animal cells. The β -cell values are considerably less than resting frog muscle (13), where $P_K = 17 \times 10^{-9} \text{ m sec}^{-1}$, and toad lens fibers

(7), where $P_K = 31 \times 10^{-9} \text{ m sec}^{-1}$. The β -cell values are much greater than that of human erythrocytes (2), where $P_K = 0.001 \times 10^{-9} \text{ m sec}^{-1}$. However, the value is comparable with that of rat liver cells (5), where $P_K = 0.76 \times 10^{-9} \text{ m sec}^{-1}$. These values must be related to the functions of the cell membrane in the various specialised cell types.

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