POTASSIUM DEPENDENCE OF THE MEMBRANE POTENTIAL OF PANCREATIC B-CELLS

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1. Introduction

In excitable tissues, like nerve and muscle, the resting membrane potential is mainly determined by the difference between intra- and extracellular concentrations of potassium [1]. In the presence of high external [K], the cells depolarize and their membrane potential approximates the K equilibrium potential [2–4]. At low external [K], however, the membrane potential deviates markedly from the K equilibrium potential because the Na permeability is not negligible.

Because non-excitable cells generally have a smaller size than nerve or muscle cells, with a higher surface: volume ratio [5], the accurate measurement of their membrane potential has long been difficult. Originally, most of these cells seemed to be characterized by a low membrane potential, tentatively explained by a high permeability ratio P_{Na}/P_K [6].

In the early recordings of Dean and Matthews [7], the membrane potential of pancreatic B-cells was low and the depolarization produced by high extracellular [K] was therefore of reduced amplitude. These observations were interpreted as evidence for a relatively high membrane P_{Na}/P_K ratio in B-cells [8] like in other gland cells [6,9]. Since much higher membrane potentials are found with our technique of continuous recording with microelectrodes [10,11], it was important to evaluate in detail the effect of extracellular [K] on the membrane potential of insulin secreting cells. This report shows that the resting membrane potential of pancreatic B-cells is mainly determined by the K permeability, whereas the Na permeability is much lower than previously suggested and provides the first estimations of intracellular Na^+ and K^+ activities for these cells.

2. Methods

All recordings were made with the microelectrode technique [10]. Single B-cells of partially microdissected islets of fed female NMRI mice were impaled with microelectrodes filled with 2 M K-citrate (tip resistance 200–300 MΩ). Membrane potential and electrical activity were continuously recorded on an oscilloscope (Tektronix 565) and an ink writer (W & W 1100). The tissue was placed in a small chamber and perifused at 37°C, at a rate of 3 ml/min; the dead time of the system is 20 s. The medium utilized was a Krebs-Ringer bicarbonate buffer, pH 7.4, gassed with O_2/CO_2 (95:5) and with the following ionic composition (mM) : Na 143; K 6; Ca 2.5; Mg 1.2; Cl 128; PO_4H_2 1.2; SO_4 1.2; CO_3H 25. As required, it was also supplemented with 2.8 or 16.6 mM glucose. When the medium [K] was increased, [Na] was isoosmotically decreased to maintain a constant [Na + K]. In the solutions containing 150 mM K, NaCl and $NaHCO_3$ were completely replaced by KCl and $KHCO_3$.

3. Results and discussion

B-cells were identified by the characteristic electrical activity [10–12] that they exhibit in the presence of a glucose concentration (16.6 mM) which

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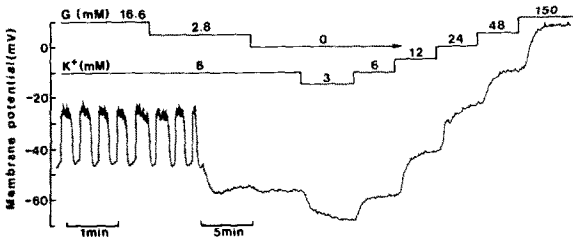

Fig.1. Effects of different [glucose] and [K] values on the membrane potential of a single B-cell. Continuous ink recording over a period of 39 min. Note different time scales. At the beginning of the experiment the preparation was perifused with 16.6 mM glucose to identify B-cells by their typical electrical activity characterized by its burst pattern. The full amplitude (~15 mV) of the fast spikes starting from the plateau potential are not recorded in this figure because of the inertia of the recording system. The short delay of the depolarizations after the changes in extracellular [K] is mainly due to the dead space of the perifusing system. The 0 potential was determined after withdrawal of the electrode from the cell.

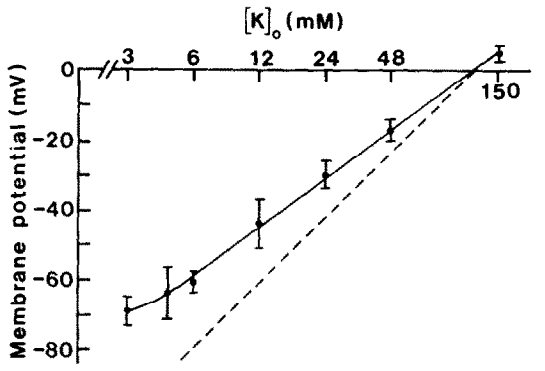

Fig.2. Relationship between the membrane potential of pancreatic B-cells and the logarithm of the external [K] in absence of glucose stimulation (0 mM or 2.8 mM glucose). The data were obtained in 10 different B-cells of 10 different mice, by an experimental procedure as shown in fig.1. Individual measurements in the presence of each [K] were made after stabilization of the membrane potential at the new level (4–5 min). Values are means ± SD. The broken line represents the theoretical change of membrane potential with a slope of 61.5 mV/10-fold change in extracellular [K].

stimulates insulin release. As shown in fig.1, this electrical activity ceased within 1 min, and the membrane hyperpolarized by about 10 mV, when the glucose concentration was decreased to a non-stimulating level (2.8 mM). Subsequent omission of glucose did not significantly change the membrane potential, whereas a further polarization was recorded when medium [K] was reduced from 6 to 3 mM. Stepwise increases in extracellular [K] produced successive depolarizations of the B-cell membrane (fig.1). Complete replacement of Na by K reversed the membrane potential to positive values (4.5 ± 1.3 mV, SD). Upon return to a control medium (6 mM K) the cells repolarized to a value similar to that initially measured in the same conditions (not shown).

Figure 2 shows the changes in resting membrane potential of B-cells at a non-stimulating glucose concentration, plotted against the extracellular [K]. Above 6 mM K, the membrane potential varied linearly with the logarithm of external [K]; the slope of this line was 47 mV for a 10-fold change in extracellular [K]. This value is markedly higher than that of 13 mV reported for B-cells [7] and also distinctly higher than that found in many non-excitable cells, except salivary acinar cells and adrenal cortex cells [6,9]. Nevertheless, it remains below the theoretical value of 61.5 mV (37°C)/10-fold change in extracellular [K], that would be expected from the Nernst equation if the B-cell membrane were selectively permeable for K^+. This deviation suggests that the resting membrane potential of B-cells depends on additional factors (other permeabilities?, pump?) and is not determined exclusively by the K permeability.

Figure 2 also shows that the membrane potential approaches 0 mV at external [K] ~120 mM. This provides an estimate of the intracellular activity of K^+ in B-cells. In addition, if one assumes that the total amount of intracellular free cations is 150 mM, an intracellular Na^+ activity of ~30 mM can be arrived at. This value is much lower than the intracellular [Na] of 95 mM measured in islet cells with a radioactive tracer [13]. The discrepancy between estimations of Na^+ activity and [Na] may suggest that a substantial proportion of Na is bound in pancreatic B-cells.

If chloride is in equilibrium with the membrane potential and does not contribute any ionic current under resting conditions [7], the permeability ratio

P_{Na}/P_K can be calculated from the modified Goldman equations (1) and (2) [14,15]:

$$RP = \frac{RT}{F} \ln \frac{P_K [K^+]_o + P_{Na} [Na^+]_o}{P_K [K^+]_i + P_{Na} [Na^+]_i} \quad (1)$$

or

$$RP = \frac{RT}{F} \ln \frac{[K^+]_o + \alpha [Na^+]_o}{[K^+]_i + \alpha [Na^+]_i} \quad (2)$$

where RP = resting membrane potential, $\alpha = P_{Na}/P_K$ and where R, T and F have their usual significance. Using the value of 120 mM for $[K^+]_i$ and assuming an intracellular Na^+ activity of 30 mM, a P_{Na}/P_K ratio of 0.043 is found. This value is similar or slightly higher than that found in various muscles or nerves [4,15–17] and shows that, in the resting state, the B-cell membrane is almost 25-times more permeable for K^+ than for Na^+.

In conclusion, the present experiments suggest that the resting membrane potential of pancreatic B-cells is mainly, although not entirely, determined by the K permeability of the plasma membrane. It is possible that the Na permeability also influences the resting membrane potential of B-cells, but the permeability ratio P_{Na}/P_K is much lower than suggested [8] and closer to that found in excitable [15–17] than in many non-excitable tissues [6,9].

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