Upsala J Med Sci 86: 171–176, 1981

On the Metaphysics of Membrane Potential in Islet Cells: Studies with Triphenylmethylphosphonium

I.-B. Täljedal

From the Department of Histology and Cell Biology, University of Umeå, Umeå, Sweden

INTRODUCTION

The membrane electric potential is in practice a useful concept in the description of excitable cells, but the precise meaning of this concept is somewhat obscure. 'Pumps' apart, the driving force for moving an ion between two physicochemical phases (say cytoplasm and extracellular fluid) is the electrochemical potential difference between the phases for the ion in question. The electrochemical potential of either phase depends on the electrical state as well as on the chemical composition of the phase. There is in general no simple relationship between the electrical state and the chemical composition that would allow an electric potential difference to be readily calculated from the ion concentrations. This is so because a general formulation of the electrochemical potential involves a third term, μ_0, that is independent of the electrical state but dependent on the chemical composition in a way not related to the ion concentration (1, 2),

$$\mu = zF\psi + RT\ln(fc) + \mu_0 \qquad \text{(I)}$$

where μ is the electrochemical potential, z is the ion charge number, F is the Faraday constant, ψ is the electric potential, R is the general gas constant, T is the thermodynamic temperature, f is the activity coefficient for the ion, and c is the concentration of the ion.

At equilibrium, when the electrochemical potential difference between two phases is zero, rearrangement of equation (I) gives a formal description of the electric potential difference,

$$E = \psi_1 - \psi_2 = -RTz^{-1}F^{-1}\ln(f_1c_1f_2^{-1}c_2^{-1}) - z^{-1}F^{-1}(\mu_{0_1} - \mu_{0_2}) \qquad \text{(II)}$$

If the two phases have identical chemical composition, then, of course,

$\underline{f}_1 = \underline{f}_2$ and $\underline{\mu_0}_1 = \underline{\mu_0}_2$, and so the electric potential difference can be described in terms of the ion concentrations,

$$\underline{E} = -\underline{RTz}^{-1}\underline{F}^{-1}\ln(\underline{c}_1\underline{c}_2^{-1}) \qquad \text{(III)}$$

Equation (III) is commonly used to compute 'membrane potentials' from measured concentrations of ions on either side of a membrane, be it a plasma membrane or the membrane of an organelle. Strictly speaking, however, since equation (III) has been derived to hold at equilibrium for phases of identical chemical composition, $\underline{c}_1$ should also equal $\underline{c}_2$, and hence equation (III) is only true with certainty for the trivial case of $\underline{E} = 0$.

Whenever it is interesting to measure membrane electric potentials, there is always a deviation from the strict identity condition relied on when deriving equation (III). Thus, the application of equation (III) may involve a certain self-contradiction that can be more or less vicious depending on how much the phases under consideration differ from eachother. For similar phases based on identical solvents, equation (III) will be the more valid the lower the ion concentrations as $\underline{f}_1$ approaches $\underline{f}_2$. In the more extreme case of different solvents, there seems to be no way of determining $(\underline{\mu_0}_1 - \underline{\mu_0}_2)$ separately from $(\underline{\psi}_1 - \underline{\psi}_2)$ in equation (II); in such a case the very concept of an <u>electric</u>, as distinct from an <u>electrochemical</u>, potential difference seems to lack definition and physical meaning (1).

It appears from the above that the concept of the membrane electric potential has a weak metaphysical status, as in principle the entity referred to may not be measurable in actual cells and organelles: the phases separated by plasma membranes typically differ in chemical composition, and it has even been suggested that they differ in solvent (4). Yet, membrane potentials are routinely measured and their exact values in volts reported. This situation constitutes an interesting paradox.

Regardless of theoretical misgivings, in empirical science concepts can solidify metaphysically be simply proving their being functional in a sufficiently diverse and varied set of situations. Up to now, membrane potentials in islet cells have been exclusively measured by micro-electrodes, and those have usually been filled with concentrated potassium citrate. Against this background it was decided to test whether the islet-cell uptake of a lipophilic cation (7), triphenylmethylphosphonium ($TPMP^+$), would be as predicted by equation (III) in conjunction with previously reported values for the membrane potential in beta-cells (5, 6). A fuller report of the experimental work is being prepared (J. Sehlin & I.-B. Täljedal, unpublished work).

METHODS

Adult non-inbred Umeå-ob/ob-mice and inbred normal NMRI mice (Anticimex, Stockholm, Sweden) were starved overnight. ob/ob-Mouse islets were isolated by free-hand microdissection, whereas normal mouse islets were isolated by collagenase digestion of the pancreas. Islets were incubated in a salt-balanced medium similar to Krebs-Ringer bicarbonate except that bicarbonate was replaced by 20 mM Hepes and that air was used as the gas phase. Triphenyl(^{3}H-methyl)-phosphonium bromide (NEN Chemicals, Dreieich, Germany) was added as required (0.37--5.6 μM) together with 0.1 mM L-(1-^{14}C)glucose as extracellular marker; non-radioactive $TPMP^{+}$ was from Sigma Chemical Co., St. Louis, MO, USA). After freeze-drying and weighing on a quartz-fibre balance, the islets were dissolved in Instafluor and Aquasol (8:2 by vol.) and analyzed for ^{3}H and ^{14}C by two-channel liquid-scintillation counting.

RESULTS AND DISCUSSION

The uptake of $TPMP^{+}$ by ob/ob-mouse islets reached an apparent equilibrium within 60 min. In incubations for 60--120 min in basal medium containing 3 mM D-glucose, the ob/ob-mouse islet-cell uptake of $TPMP^{+}$ was 20-fold with 0.37 μM $TPMP^{+}$ in the medium, 30-fold with 1.4 μM, 60-fold with 2.8 μM, 50-fold with 5.6 or 20 μM, 30-fold with 200 μM, and 20-fold with 1000 μM; uptake by normal mouse islet-cells was also 50-fold with 5.6 μM $TPMP^{+}$.

Conjoined with equation (III) the membrane potential recorded for resting beta-cells by measurements of electromotive force (5, 6), -60 mV (inside negative), predicts a 10-fold accumulation of passively distributed cations. Thus, the islet-cells accumulated much more $TPMP^{+}$ than predicted. The question as to how the uptake values might be plausibly adjusted was therefore considered. Firstly, after pretreating the islets with excess (50 μM) non-radioactive $TPMP^{+}$, the uptake of (^{3}H)$TPMP^{+}$ was suppressed to about 20--25-fold (5.6 μM in medium). This effect might reflect short-circuiting of the plasma membrane and abolishment of any membrane electric potential, the prevention of (^{3}H)$TPMP^{+}$ binding to high-affinity sites, metabolic arrest, or any combination of such factors. The residual (^{3}H)$TPMP^{+}$ uptake after pretreatment with 50 μM non-radioactive $TPMP^{+}$ was therefore tentatively considered non-specific and irrelevant for the membrane potential. Secondly, when islets had been preloaded with (^{3}H)$TPMP^{+}$ (5.6 μM in medium), their loss of label approached an asymptot corresponding to about 70 μmol $TPMP^{+}$/kg dry islet. This value was tentatively considered to represent high-affinity binding without relevance for the

membrane potential.

The two modes of adjusting the uptake data are illustrated in Fig. 1, which summarizes the effects of extracellular K^+ on the $TPMP^+$ uptake. To facilitate comparison with micro-electrode recordings (5), the uptake values are expressed in terms of electric potentials calculated according to equation (III). Because $^{42}K^+$ equilibration experiments have indicated that the present type of islet cells contain about 165 mM K^+ internally when incubated with 5.9 mM K^+ in the presence of 3 mM D-glucose (J. Sehlin & I.-B. Täljedal, unpublished work), one expects complete depolarization of the cells around 165 mM K^+ in the medium. Unadjusted $TPMP^+$ uptake values were very far from meeting this expectation (Fig. 1). However, both methods for adjusting the $TPMP^+$ uptake data made the calculated potential more sensitive to K^+ within the range 5.9--130 mM, and the first method even appeared compatible with complete depolarization around 165 mM K^+. Regardless of how the data were adjusted, the membrane potential estimates did not vary in the range 1.2--5.9 mM K^+. This stability is reminiscent of measurements by electrodes in that range (5). It contrasts with potential estimates calculated from $^{86}Rb^+$ uptake data (J. Sehlin & I.-B. Täljedal, unpublished work), suggesting that $TPMP^+$ is neither qualitatively nor quantitatively a mere analogue of K^+ in the islet cells.

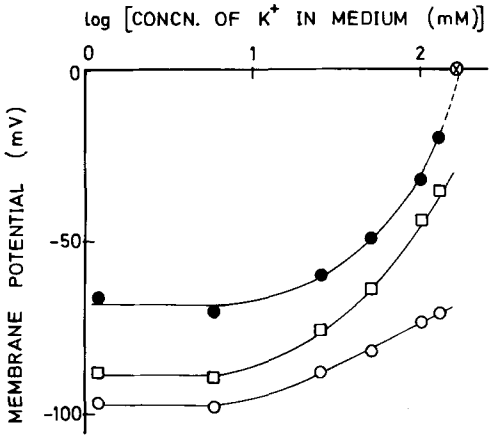

Fig. 1. Effect of extracellular K^+ on E (equation III) as calculated from the $TPMP^+$ uptake by ob/ob-mouse islet cells (5.6 µM in medium). All values are corrected for $TPMP^+$ in the extracellular space. Open circles = no further adjustment of data; solid circles = adjustment for binding according to first method; open squares = adjustment for binding according to second method. The circle with a cross indicates the logarithm of 165 which in mM is the K^+ concentration recorded by $^{42}K^+$ equilibration in ob/ob-mouse islet cells.

Fig. 2 shows that the islet uptake of $TPMP^+$ was also inhibited by 20 mM D-glucose. This effect of D-glucose was in its turn prevented by 20 mM D-mannoheptulose, an inhibitor of glucose-stimulated insulin release. In contrast to D-glucose, 3-O-methyl-D-glucose, which has no effect on insulin release, lacked effect on the $TPMP^+$ uptake (not shown). Against this background it seems possible that the changes of $TPMP^+$ uptake reflect such changes of the intracellular ionic milieu as are important for the regulation of insulin release. The effect of D-glucose was reproduced with ob/ob-mouse islets in 0.37 or 1.4 µM $TPMP^+$ and with normal NMRI-mouse islets in 5.6 µM $TPMP^+$.

The uptake of $TPMP^+$ was markedly inhibited by the metabolic poison, 0.5 mM 2,4-dinitrophenol (Fig. 2), whereas the fluorescent calcium probe, 10 μM chlorotetracycline (8, 9), had no effect (not shown). Neither glucose nor dinitrophenol had any effect on the residual uptake of $(^3H)TPMP^+$ after pre-treating the islets with 50 μM non-radioactive $TPMP^+$.

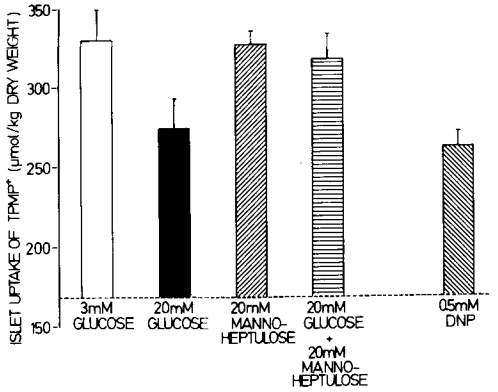

Fig. 2. Effects of D-glucose, D-mannoheptulose, and dinitrophenol (DNP) on the $TPMP^+$ uptake in 60 min by ob/ob-mouse islets (5.6 μM in medium). Values are corrected for $TPMP^+$ in the extracellular space. Mean values ± S.E.M. for 9--18 different experiments. The broken horizontal line indicates $(^3H)TPMP^+$ uptake after pre-treating the islets with 50 μM non-radioactive $TPMP^+$.

In qualitative terms, the effects of K^+, D-glucose, and D-mannoheptulose on $TPMP^+$ uptake paralleled those on the membrane electric potential as measured by micro-electrodes. In quantitative terms, the membrane potentials calculated from equation (III) and the $TPMP^+$ uptake required substantial corrections of the uptake values in order to match the values recorded by electrodes. As those corrections appear rather natural, the results can be said to support the practice of regarding the membrane electric potential as a non-arbitrary entity. Yet, it is unsatisfactory that there is no obvious way of calculating on beforehand how much uptake of $TPMP^+$ should be expected because of voltage-independent binding. To divide the $TPMP^+$ uptake into one voltage-independent and one voltage-dependent part would seem question-begging to the extent that it represented an arbitrarily high degree of microscopic dissection of the intracellular physico-chemical phase, akin to the questionable subdivision of the electrochemical potential.

Finally, it should be mentioned that dispersed islet cells in suspension (3) took up $TPMP^+$ to about the same extent as the cells in whole islets, indicating that the cells are not abnormally depolarized as a result of the dispersion technique (not shown). The validity of the dispersed cells as an experimental model for specific purposes (e.g. 3, 8, 9) was also supported by their adequate responsiveness to K^+, which suppressed $TPMP^+$ uptake in much the same way as in whole islets. Electrodes have not yet been used to measure the membrane electric potential in dispersed islet-cells.

ACKNOWLEDGEMENTS

This work was part of a project supported by the Swedish Medical Research Council (12x-2288), the Swedish Diabetes Association, and Novo Industri AB.

REFERENCES

1. Guggenheim, E.A.: Thermodynamics. An Advanced Treatment for Chemists and Physicists. North-Holland Publishing Company, Amsterdam, 1967.
2. Kotyk, A. & Janáček, K.: Cell Membrane Transport. Principles and Techniques. Plenum Press, New York-London, 1970.
3. Lernmark, Å.: The preparation of, and studies on, free cell suspensions from mouse pancreatic islets. Diabetologia 10: 431--438, 1974.
4. Ling, G.N.: The physical state of water and ions in living cells and a new theory of the energization of biological work performed by ATP. Mol Cell Biochem 15: 159--172, 1977.
5. Meissner, H.P., Henquin, J.C. & Preissler, M.: Potassium dependence of the membrane potential of pancreatic B-cells. FEBS Lett 94: 87--89, 1978.
6. Meissner, H.P., Preissler, M. & Henquin, J.C.: Possible ionic mechanisms of the electrical activity induced by glucose and tolbutamide in pancreatic islets. In: Diabetes (ed. W.K. Waldhäusl), pp. 166--171. Excerpta Medica, Amsterdam, 1979.
7. Schuldiner, S. & Kaback, H.R.: Membrane potential and active transport in membrane vesicles from Escherichia coli. Biochemistry 14: 5451--5461, 1975.
8. Sehlin, J. & Täljedal, I.-B.: $^{45}Ca^{2+}$ uptake by dispersed pancreatic islet cells: effects of D-glucose and the calcium probe, chlorotetracycline. Pflügers Arch 381: 281--285, 1979.
9. Täljedal, I.-B.: Polarization of chlorotetracycline fluorescence in pancreatic islet cells and its response to calcium ions and D-glucose. Biochem J 178: 187--193, 1979.