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A NOVEL METHOD FOR THE DETERMINATION OF ELECTRICAL POTENTIALS ACROSS CELLULAR MEMBRANES

I. THEORETICAL CONSIDERATIONS AND MATHEMATICAL APPROACH *

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Summary

A new method for the determination of electrical potentials across cellular membranes has been developed. In order to determine the membrane potential, cells were incubated in buffer solutions with increasing concentrations of KCl. Parallel experiments were performed with buffer solutions which additionally contained valinomycin. After sedimentation of the cells, the membrane potential was calculated from data which were obtained by simply measuring the wet mass, the dry mass and the potassium content of cell pellets by atomic absorption spectroscopy.

Introduction

The electrical potential across cellular membranes plays an important role in many physiological functions by controlling ion currents, performing energy transduction and mediating excitation propagation in nerves [1]. Therefore, knowledge of the transmembrane electrical potential is an essential prerequisite in understanding membrane-bound biological functions.

Different techniques have been developed to investigate membrane potentials. Membrane potentials of cells which can be impaled with microelectrodes are measured directly using electrophysiological techniques [2]. Membrane potentials of cells which are too small to permit the use of microelectrodes can be determined by following several techniques. A current method is the estima-

* Our paper is dedicated to Professor Wilhelm Hanle on his 80th birthday.

tion of the passive distribution of Cl^- ($^{36}Cl^-$) between the interior and exterior of the cells [3].

For membranes which are practically impermeable to K^+, at least under physiological conditions, the contribution of the potassium diffusion potential to the total membrane potential is negligible. When such membranes are rendered selectively permeable to K^+, the potassium distribution equals the Donnan equilibrium distribution. Thus, the membrane potential is given by the equation:

$$\psi = (RT/F) \cdot \ln(K_{out}/K_{int}) \quad (1)$$

where K_{out} is the extracellular and K_{int} the intracellular potassium concentration, respectively.

For a definite intracellular potassium concentration ($K_{int,o}$), there exists a definite ('critical') extracellular potassium concentration, $K_{out} = K_{crit}$, so that in this special case the K^+ distribution equals the Donnan equilibrium distribution. Addition of the potassium-selective carrier valinomycin to cells suspended in a medium with the critical potassium concentration, K_{crit}, results in no net flow of potassium across the membrane, i.e., the potassium concentration inside the cells remains constant. The determination of membrane potential using potential-sensitive fluorescent dyes is based on these facts. By applying these dyes which had recently been developed by Waggoner [4,5], the critical potassium concentration, K_{crit} (also referred to as 'null point concentration' [6]), can be determined. If the internal potassium concentration of the native cells, $K_{int,o}$, is known, the membrane potential can be calculated according to Eqn. 1.

In order to determine the intracellular potassium concentration, $K_{int,o}$, some rather troublesome measurements must be performed. The total potassium content, K_{tot}, of a weighed amount of cells is determined by, for example, flame photometry. The total intracellular water volume, $V_{int,tot}$, is calculated from the data of the dry and wet mass of the cells. The extracellular water volume is determined by labeling the extracellular water space with a marker, e.g., tritium dextran or $Na_2{}^{35}SO_4$ which cannot penetrate the cell membrane; after sedimentation and careful removal of the supernatant, the extracellular water volume is calculated by measuring the radioactivity of the sediment.

This collection of different and rather complicated methods for the determination of membrane potentials can be overcome by our method which is especially useful when potential-sensitive dyes cannot be applied due to their toxicity.

Our method is based on determinations of potassium concentrations and gravimetric measurements. Furthermore, it is an excellent method for determining the extracellular water volume of a sedimented cell pellet.

Mathematical Approach

Cells are incubated in a medium containing a known potassium concentration, K_{out}. After centrifugation the supernatant is removed. The potassium concentration of the extracellular water volume of the pellet equals the potassium concentration of the medium since $V_{medium} >> V_{ex}$. This procedure will not

change the initial internal potassium concentration, $K_{int,o}$, of non-metabolizing cells with a membrane impermeable to K^+. After disintegration of the cells in a potassium-free medium, e.g., 0.1 M hydrochloric acid, with the volume V_L, the potassium concentration in the solution is:

$$K(K_{out}) = \frac{V_{ex}}{V_L + V_{tot}} \cdot K_{out} + \frac{V_{int}}{V_L + V_{tot}} \cdot K_{int,o} \quad (2)$$

where V_L is the volume of the disintegrating solution; V_{ex}, the extracellular and V_{int} the intracellular water volume, respectively; V_{tot}, the total pellet water volume; $K_{int,o}$, the intracellular potassium concentration of native cells; and K_{out}, the potassium concentration of the incubation medium.

For routine experimental conditions, $V_L = 1$ ml and $V_{tot} \leqslant 10$ µl, i.e., $V_L >> V_{tot}$, and therefore Eqn. 2 is reduced to:

$$K(K_{out}) = \frac{V_{ex}}{V_L} \cdot K_{out} + \frac{V_{int}}{V_L} \cdot K_{int,o} \quad (3)$$

Let us assume that aliquots of cells are incubated in media containing different potassium concentrations, K_{out}, and treated as described above. A plot of the measured potassium concentration $K(K_{out})$ vs. K_{out} shows a linear relationship between $K(K_{out})$ and K_{out}. The slope of the resulting straight line is: $s = V_{ex}/V_L$. Thus, we can calculate the external water volume, V_{ex}, from the slope of the curve according to $V_{ex} = sV_L$.

The intracellular water volume, V_{int}, is determined as follows: The total mass (m_{ww}, wet weight) and, after drying the cell pellet in vacuo, the dry mass (m_{dw}, dry weight) are measured. The terms are related by:

$$m_{ww} = V_{ex} \cdot \rho_{ex} + V_{int} \cdot \rho_{int} + m_{dw} \quad (4)$$

where ρ_{ex} and ρ_{int} denote the density of the extra- and intracellular fluid, respectively. The densities are assumed to equal the density of water since, for example, the density of a 300 mM KCl solution is only 1.0022 times the density of water. Then we can use the notation:

$$\rho_{ex} = \rho_{int} = \rho_{H_2O} = \rho$$

Hence, Eqn. 4 is reduced to:

$$m_{ww} = (V_{ex} + V_{int}) \cdot \rho + m_{dw} \quad (5)$$

and we obtain:

$$V_{int} = (m_{ww} - m_{dw}) \cdot \rho^{-1} - V_{ex} \quad (6)$$

Since all terms on the right-hand side of Eqn. 6 are known, we can calculate the intracellular water volume, V_{int}.

The intracellular potassium concentration, $K_{int,o}$, can be calculated from the intersection point of the curve with the ordinate. For $K_{out} = 0$ we have:

$$K(0) = \frac{V_{int}}{V_L} \cdot K_{int,o} \quad \text{and} \quad K_{int,o} = \frac{V_L}{V_{int}} \cdot K(0) \quad (7)$$

Some organisms when incubated in potassium-free media, lose part of their cellular potassium. By using our method, incubation of organisms in potassium-

free medium can be avoided because $K_{int,o}$ is found by simple extrapolation.

If valinomycin, a potassium-selective ion carrier [7], is added to the organisms in media containing different potassium concentrations, K^+ will be distributed according to the Donnan equilibrium. If the volume of the incubation medium is much greater than that of the intracellular water space (this assumption holds true under routine experimental conditions), the potassium concentration of the medium does not alter upon addition of valinomycin. Therefore, in the Donnan equilibrium the potassium concentration will be $K_{out}/K_{int} = d$ = constant, i.e., K_{int} depends upon the potassium concentration of the incubation medium: $K_{int} = K_{out}/d$. Specimens which were prepared as described above, have a potassium concentration given by:

$$K^v(K_{out}) = \frac{V_{ex}}{V_L} \cdot K_{out} + \frac{V_{int}}{V_L} \cdot \frac{K_{out}}{d} \tag{8}$$

As we have shown above, there exists a potassium concentration of the incubation medium, $K_{out} = K_{crit}$, at which the addition of valinomycin does not alter the internal potassium concentration, i.e., $K_{int} = K_{int,o}$. We can express d in terms of these special values: $d = K_{crit}/K_{int,o}$, and rewrite Eqn. 8:

$$K^v(K_{out}) = \frac{V_{ex}}{V_L} \cdot K_{out} + \frac{V_{int}}{V_L} \cdot \frac{K_{int,o}}{K_{crit}} \cdot K_{out} \tag{9}$$

Let us assume that both curves, $K(K_{out})$ and $K^v(K_{out})$, have an intersection point with the abscissa $K_{out} = K'$. For this point we have: $K(K') = K^v(K')$, i.e.:

$$\frac{V_{ex}}{V_L} \cdot K' + \frac{V_{int}}{V_L} \cdot K_{int,o} = \frac{V_{ex}}{V_L} \cdot K' + \frac{V_{int}}{V_L} \cdot \frac{K_{int,o}}{K_{crit}} \cdot K' \tag{10}$$

After a simple transformation we obtain: $K' = K_{crit}$, i.e., the abscissa of the intersection point of the curves equals the critical potassium concentration. The membrane potential can then be calculated according to:

$$\psi = (RT/F) \cdot \ln\left(\frac{K_{crit}}{K_{int,o}}\right).$$

Conclusions

The theoretical basis of a novel technique to determine the membrane potential of cells has been developed. In addition, this technique allows the determination of the extra- and intracellular water volumes of centrifuged cell pellets with high accuracy. Our technique can be applied to all cells which are: (i) impermeable to K^+, at least under physiological conditions, i.e., the contribution of the potassium diffusion potential to the total membrane potential is negligible; (ii) susceptible to valinomycin; and (iii) stop K^+ transport when removed from nutrient solution. These prerequisites are met by most organisms. Some applications of our technique are given in Ref. 8.

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