Measurement of the sodium membrane potential by NMR

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Using nuclear magnetic resonance (NMR), we have developed a method of noninvasively determining the transmembrane sodium potential in erythrocytes by measuring intracellular and extracellular sodium concentrations. The experimental values correlated well with values obtained from standard flame photometric methods.

NMR *Membrane potential* *Paramagnetic shift reagent*

1. INTRODUCTION

Ionic gradients and membrane potentials are of fundamental importance in cell physiology, and changes in them have been implicated in a variety of diseases [1,2]. Unfortunately, most methods that have been devised for the measurement of these parameters, including microelectrode impalement [3], fluorescent dye permeation [4], flame photometry [5], and proton gradient/flux measurement techniques [6], are invasive and destructive. The possibility of using nuclear magnetic resonance spectroscopy (NMR) as a non-invasive, non-destructive method for estimating transmembrane potentials is therefore very attractive.

Recently, NMR has been used as a method to investigate transmembrane steady states [7–13]. To resolve intracellular and extracellular compartments, impermeable paramagnetic shift reagents such as dysprosium tripolyphosphate – $Dy(PPP)_2$ – have been employed. These reagents remain outside of cells and selectively affect the extracellular space, allowing differentiation of NMR signals arising from the two compartments. However, for a calculation of ion concentration, these previous studies have relied on rough estimates of cell water content.

Here, we present a method which allows us to quantitatively differentiate between intracellular and extracellular sodium and water of erythrocytes, thus enabling us to determine the intracellular sodium concentration and to calculate the transmembrane sodium potential.

2. MATERIALS AND METHODS

Freshly drawn heparinized blood samples from apparently healthy donors (BEC and DYS) were centrifuged at 5000 × *g* for 10 min and washed twice with an isotonic 5 mM phosphate buffer (pH 7.4) containing 10 mM glucose, 135 mM Na^+, 5 mM K^+ and 130 mM Cl^-. Following the first wash, the resuspended cells were oxygenated by passing humidified O_2 over the suspension. The cells were rewashed and then resuspended at an approximate hematocrit of 50% in an isotonic 5 mM phosphate buffer containing 5 mM $Dy(PPP)_2$, 10 mM glucose, 40 mM sorbitol, and 20% (v/v) D_2O, adjusted without isotopic correction to pH 7.4. The final concentrations of sodium and potassium were 135 mM and 5 mM, respectively. $Dy(PPP)_2$ was prepared according to [8]. Red blood cell suspensions were stored at 4°C for no longer than 3 h, and were reoxygenated and allowed to equilibrate to room temperature (25°C) before each data acquisition.

^{23}Na spectra of the cell suspensions were obtained at 25°C from a 10-mm sample tube in a Varian XL-100 spectrometer equipped with a Nicolet Instrument Corporation MONA accessory probe and 1180 data collection system. D_2O was

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used for field frequency locking; 5000 scans were collected over a 30-min period with a sweep width of ± 5000 Hz. The relative amounts of sodium ions inside and outside the cells were calculated by comparing the intensities of nonshifted and shifted peaks. To determine the effective $Dy(PPP)_2$ concentration of the erythrocyte suspensions, $Dy(PPP)_2$ titrations on standard NaCl solutions were done under similar experimental conditions with a 5-mm tube containing the buffer with no shift reagent, coaxially aligned inside a 10-mm tube containing the $Dy(PPP)_2$ buffer. ^1H spectra were obtained at 25°C from a spinning 5-mm sample tube in a Bruker HXS-360 spectrometer equipped with a Nicolet 1180 data collection system. Twelve scans were collected over 30 s with a sweep width of ± 2000 Hz. A sealed capillary tube filled with deionized water was fixed coaxially in the 5-mm tube and used as a reference.

The proportions of intra- and extracellular water were calculated from the following formula:

$$\alpha_o = \frac{\delta_i - \delta_{obs}}{\delta_i - \delta_D}, \quad \alpha_i = 1 - \alpha_o \qquad (1)$$

where α_o = fraction of total water which is extracellular, δ_{obs} = observed chemical shift of RBC suspended in $Dy(PPP)_2$ buffer relative to water reference, δ_i = chemical shift of 100% Hct RBCs relative to reference, δ_D = chemical shift of $Dy(PPP)_2$ buffer relative to water reference and corrected for 'effective' $Dy(PPP)_2$ concentration from titration curve.

Intracellular sodium concentrations were calculated from the following formula:

$$(Na_{in}/Na_{out})/\alpha_i \times 135 \text{ mM} \qquad (2)$$

where Na_{in} = the intensity of the nonshifted sodium peak, and Na_{out} = the intensity of the shifted sodium peak. The resuspension medium contained 135 mM Na^+.

The transmembrane sodium potential was calculated from a modified Nernst equation:

$$E_{Na+} = (RT/zF)\ln([Na]_{out}/[Na]_{in}) \qquad (3)$$

where R = gas constant, T = absolute temperature, F = Faraday's constant, z = charge, $[Na]_{out}$ = extracellular sodium concentration, and $[Na]_{in}$ = intracellular sodium concentration.

For comparison, intracellular sodium concentrations were also determined by flame photometry as described by Funder and Wieth [14]. Student's t-test was used for statistical analysis.

3. RESULTS

^{23}Na spectra of erythrocytes resuspended in the $Dy(PPP)_2$ buffer show two resonances with a chemical shift separation of 12 ppm, with line

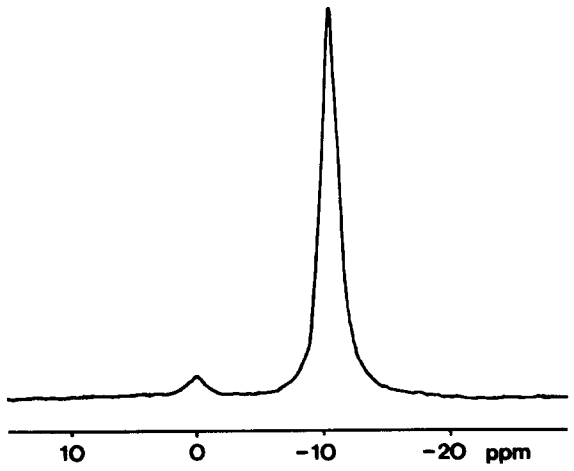

Fig.1. ^{23}Na spectrum of red blood cells in a suspension medium containing 5 mM $Dy(PPP)_2$. δ = 12 ppm. Half-height line widths for intracellular (i) and extracellular resonances (o) are 44 Hz and 73 Hz, respectively.

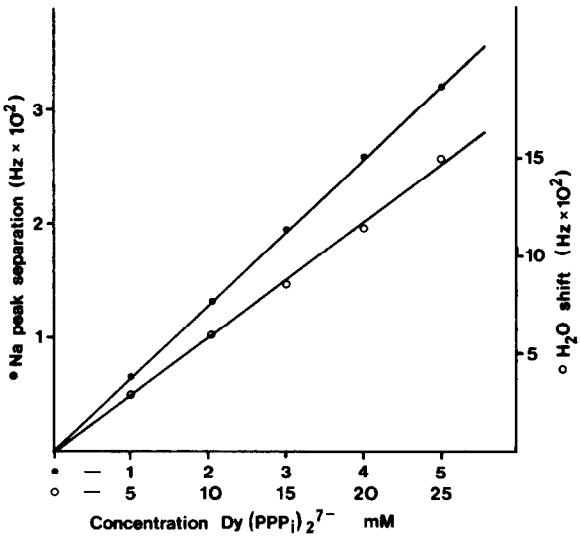

Fig.2. Chemical shift as a function of the concentration of shift reagent $Dy(PPP)_2$: (●) separation of peaks of ^{23}Na spectra, (○) shift of the single broad H_2O peak relative to deionized H_2O in coaxial capillary.

widths of 44 Hz for the unshifted peak, and 73 Hz for the shifted peak (fig.1). In both the ^{23}Na and ^{1}H spectra, the chemical shift induced by the shift reagent was found to vary linearly with the concentration of the reagent (fig.2).

While there was a clear separation of peaks for intra- and extracellular sodium, the two populations of water were not resolved in this manner in ^{1}H spectra. Water exchanges too rapidly relative to its chemical shift so that only one single broad peak is observed intermediate between the expected locations of shifted and unshifted resonances (fig.3). The chemical shift of this peak

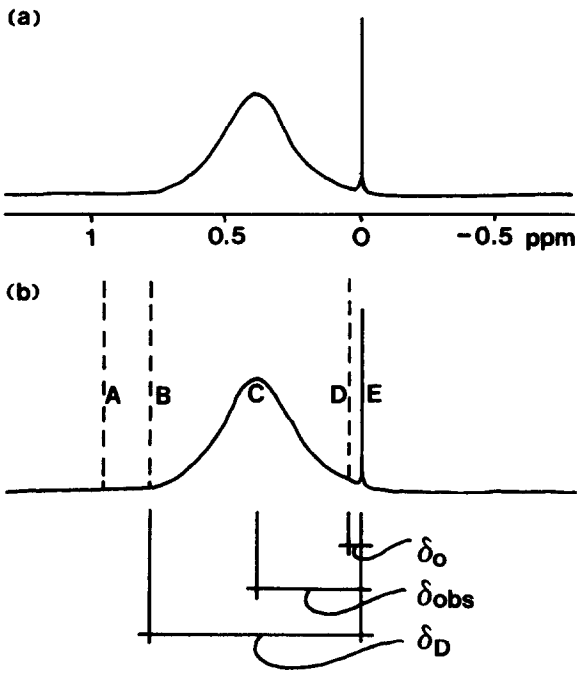

Fig.3. (a) ^{1}H spectrum of red blood cells in 5 mM $Dy(PPP)_2$, with deionized H_2O in capillary for reference. Half-height line width is 170 Hz, δ = 145 Hz. (b) Peaks C and E correspond to those in fig.3a. Peak A lies at the chemical shift of suspension buffer containing 5 mM $Dy(PPP)_2$. Peak B is the expected shift of the extracellular buffer in an RBC suspension. It differs from A due to residual extracellular shift reagent-free buffer in packed RBCs. Addition of 5 mM $Dy(PPP)_2$ buffer to packed cells results in a final extracellular concentration of shift reagent of less than 5 mM. The exact concentration is calculated from the chemical shift separation of the ^{23}Na peaks. Peak D corresponds to the shift of H_2O in RBCs in the absence of shift reagent. δ_o, δ_{obs}, and δ_D are explained in the text under (eq.1).

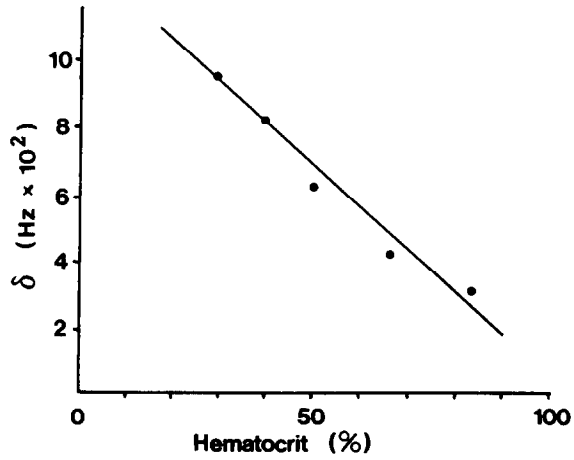

Fig.4. Chemical shift of ^{1}H peak relative to deionized H_2O as a function of hematocrit. $[Dy(PPP)_2]$ = 21 mM.

was found to be dependent on the hematocrit (fig.4).

From the measurements of the relative proportions of sodium and water inside and outside of cells, the calculated value for the intracellular sodium concentration was found to be 10.7 ± 1.9 meq/l (mean ± SD, n = 6). This translates to a sodium potential of +65 mV. The corresponding value from the flame photometric determination was 12.5 ± 1.1 meq/l (mean ± SD, n = 5).

4. DISCUSSION

The ability to measure the membrane potential of erythrocytes, nerve, muscle, and other tissue in vivo would further the understanding of their physiology, and may aid in the diagnoses of disorders. Alterations in intracellular sodium concentrations have been noted in such conditions as non-respiratory alkalosis, uremia, muscular dystrophy, hypertension, cystic fibrosis, affective disorders, acute myelogenous leukemia, hyperthyroidism, digoxin therapy ([1] and references therein), and cancer [2].

Erythrocytes are ideally suited for the validation of this NMR method because they are easily accessible, are a relatively homogeneous cell population, lack intracellular compartments, are easily aliquoted and diluted, and have well characterized transport systems. Thorough oxygenation of the venous blood-derived erythrocytes was necessary

in this study to minimize the paramagnetic contributions of deoxyhemoglobin [15].

Our mean value of 10.7 meq/l correlates well with the value for intracellular sodium as determined by flame photometry measurement ($p > 0.10$). The range of published normal values for erythrocytes is large (6.2–16.6+ meq/l) ([5,16] and references therein), reflecting different assays used in various laboratories.

The close correlation of the mean NMR value and the mean flame photometry value also suggests that all of the intracellular sodium is NMR-visible, in agreement with Pettegrew et al. [13]. This differs from the results of Ogino et al. [12] using *Saccharomyces*, which suggested that only 40% of the intracellular sodium is NMR-visible in that system.

Although the potassium potential and the chloride potential correlate with the total transmembrane potential more closely than does the sodium potential, sodium has the advantage of a higher intrinsic NMR sensitivity, and measuring the sodium potential does provide a measure of the functional integrity of the cell membrane.

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