Potassium-Specific Ion-Exchanger Microelectrodes to Measure K⁺ Activity in the Renal Distal Tubule

FRED S. WRIGHT and W. SCOTT McDOUGAL

Department of Physiology, Yale University School of Medicine, New Haven, Connecticut 06510

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The recent introduction of miniature liquid ion-exchanger electrodes provides the possibility of overcoming previously encountered limitations on the measurement of specific ionic activities in biological fluids. Hydrogen ion and sodium ion activities have been successfully determined by means of glass-membrane electrodes(1–5). However, in the presence of sodium, glass electrodes are usually inadequate for measuring potassium, divalent cation or anion activities(4). The adaptation of the liquid ion-exchanger principle for use with microelectrodes, permits measurement of K⁺ and Cl⁻ activities on both sides of the cell membrane(6–8).

We have constructed potassium-selective liquid ion-exchanger microelectrodes to measure K⁺ activity in rat distal tubules. Because this technique is still in a relatively early stage of development, it may be useful to review some details of the theoretical basis for the method and some of the practical considerations relevant to its application. Our experience has been with K⁺ ion-exchanger electrodes but the same techniques apply to anion-sensitive electrodes.

Liquid ion exchangers are solutions in an organic solvent of a charged organic compound(9). Such ion-exchanger solutions were originally developed for industrial purposes; in the early 1960's they were increasingly studied as model membrane systems having many of the properties of carrier-mediated ion permeation(9–11). A Ca²⁺ selective macroelectrode was described by Ross(12), and

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Wright, Kurey, and Baum(13) constructed a K+ selective macroelectrode. Subsequently the K+ ion exchanger was employed in microelectrodes by Walker and Brown(6,7) and also by Khuri, Agulian, and Wise(8).

A physical property required of both the solvent and the ion-exchanger compound is a very low water solubility so that the exchanger solution can form a membrane-like phase separating two aqueous electrolyte solutions(10,11). Solvents that have been used have low dielectric constants(14). The high degree of association between solvent molecules tends to exclude polar compounds, both water and ions. Some of the solvents that have been used in liquid ion-exchanger systems are xylene(10), diphenyl ether(15), nitrobenzene(10,11), bromobenzene (15), chlorobenzene(15), and 1,2-dichlorobenzene(l1). In general, these solvents have viscosities similar to water, are more dense than water, and have high boiling points thus are relatively nonvolatile. The commercially available exchanger that we have used (Corning No. 477317) employs 1-2-dimethyl-3-nitrobenzene to dissolve 2g/100 ml potassium tetrakis (p-chlorophenyl) borate(16).

When the organic solution contacts an aqueous electrolyte solution, several reactions have been postulated to occur(4). Any cation I+ dissolves sparingly in the membrane phase and exists there in its dissociated form I+ (the asterisk denotes existence in the membrane phase). The position of this reaction at equilibrium is shifted far to the left and is described by the partition coefficient, ki.

\[ \text{I}^+ \overset{k_i}{\Rightarrow} *\text{I}^+ \]  

(1)

Most of the cations in the membrane phase associate with mobile sites, S−, in this case the negatively charged chloride groups on the exchanger molecule, to form a neutral compound IS. This equilibrium favors formation of the neutral species and is described by the association coefficient, K_{IS}.

\[ *\text{I}^+ + \text{S}^- \overset{K_{IS}}{\Rightarrow} *\text{IS} \]  

(2)

When two cationic species are present in the aqueous solution, both enter the partition and association reactions and reach an equilibrium.

\[ *\text{J}^+ + *\text{IS} \overset{K_{ij}}{\Rightarrow} *\text{JS} + *\text{I}^+ \]  

(3)

Whether a larger fraction of the associated form is in the IS form or the JS form is described by the ion-exchange equilibrium coefficient, K_{ij}. This number is theoretically dependent only on the ratios of the partition coefficients and the association coefficients for the two ions.

\[ K_{ij} = \frac{k_j \cdot K_{IS}}{k_i \cdot K_{IS}} \]  

(4)

Thus, properties of both the solvent and the mobile sites may determine the relative selectivity of the ion exchanger for different cations. K_{ij} is considered numerically equal to the empirically determined selectivity coefficient.
A schematic physical description of the ion exchanger–aqueous solution boundary and its incorporation into an electrode is illustrated in Fig. 1. The organic anions are confined to the ion-exchanger phase. Cations in the test solution enter the organic phase and associate with the negative sites. Anions in the test solution are excluded from the ion-exchanger system. Because cations enter and anions are excluded, an electrical potential difference is established at this boundary that is dependent on the cation concentration in the test solution. The magnitude of the electrical potential is proportional to the affinity of the ion-exchanger system for the cation. A smaller concentration of a cation species for which the exchanger has a high affinity can give the same potential as given by a larger concentration of another cation for which the affinity is lower.

The generation of this boundary potential, $V_2$, can be considered a half-cell reaction. When a complete circuit is established as shown in Fig. 1, the overall electrical potential difference is a function of the salt concentrations in the test and inside solutions and the types of cations in the solutions. The difference between the two boundary potentials, $V_2$ and $V_3$, can be considered to account for the total potential difference across the ion-exchanger. When the inside solution is kept constant, only changes in the test solution actually change the total circuit potential.

A quantitative theory based on the assumptions of partition and association has been developed to describe these potentials(17,18). It predicts the kind of electrode behavior that is observed experimentally(7,8,12). The electrical potential depends on the effective anion impermeability of the ion-exchanger systems. At equilibrium, a membrane permeable solely to cations and separating two solutions of a single salt generates a potential given by the Nernst equation

$$E = \frac{RT}{F} \ln \frac{a_{i^+}}{a_{i^+}'}$$

(5)

where $E$ is the membrane potential, $R$ is the gas constant, $T$ is the absolute temperature, $F$ is the Faraday, $a_{i^+}$ is the activity for the cation $I^+$ in the outside solution, and $a_{i^+}'$ is the activity in the inside solution. When the membrane is incorporated into an electrode circuit, the concentration of the salt is held constant on one side, and an aqueous liquid junction separates the reference Ag-AgCl

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**Fig. 1.** Schematic illustration of a liquid ion-exchanger membrane in a circuit. $V_1$ and $V_4$ are the potentials established by the Ag-AgCl half-cell reactions. $V_3$ and $V_5$ are shown as boundary potentials established by the ion-exchange reaction.
electrode from the test solution, then the equation for the total potential simplifies to the following.

\[ E = E_0 + \frac{RT}{F} \ln a_v \]  

(6)

\( E_0 \) is a constant (volts) depending largely on the inside solution. Variations in the activity of ion \( 1^+ \) in the test solution change the electrode potential. When more than one cationic species are present in the test solution, the degree to which variations in \( 1^+ \) affect the potential that would have been established by \( 1^+ \) alone depends on the selectivity coefficient, \( K_{ij} \). The potential of an electrode circuit with two cationic species in the test solution can be described by

\[ E = E_0 + \frac{nRT}{F} \ln (a_i + K_{ij} a_j) \]  

(7)

In practice, solutions of known activity are used to determine \( K_{ij} \) for each electrode and this number is taken to be equal to the theoretical ion-exchange equilibrium coefficient(7,12). Another empirically determined dimensionless fractional term, \( n \), is required to describe the electrode behavior because the slope of the line relating activity and potential is usually slightly less than the theoretically expected 59 mV at 25°.

After a calibration line has been obtained for a particular electrode, the electrode may be inserted into a cell or across an epithelial wall. The change in the circuit potential that is observed is described as follows(7).

\[ \Delta E = EM + \frac{nRT}{F} \ln \left[ \frac{a_{i}^\circ + K_{ij} a_{j}^\circ}{a_{i}^\circ + K_{ij} a_{j}^\circ} \right] \]  

(8)

The change in potential is the sum of the membrane potential, \( EM \), and of the change in electrode potential due to the change in cation activities at the electrode tip. Both \( \Delta E \) and \( EM \) are measured and the outside cation activities are known. \( K_{ij} \) and \( n \) are assumed to remain constant and the previous calibration is thus used to find the cation activities inside the cell or tubule. Uncertainty due to interfering cations depends on the selectivity ratios and on the activities of those ions inside the cell or tubule. A combination of high selectivity for potassium over interfering cations and low activities of those ions is ideal but may not be achieved to the extent we would wish in distal tubule measurements. Data available for the order and magnitude of these relative selectivities for several cations are shown in Table 1. The selectivity coefficients shown are ratios of the response to potassium over the response to the listed cation at concentrations of 100 mM. The electrode response to \( K^+ \) is greater than to \( NH_4^+, H^+, Na^+, Ca^{++}, Li^+, \) and \( Mg^{++} \). The response to \( Cs^+ \) and \( Rb^+ \) is greater than that to \( K^+ \). In general, there is a decreasing electrode response with decreasing size and mobility of the ion in aqueous solution. From these selectivity ratios, and the range of concentrations of the ions in biological fluids, the possible errors in measurements of \( K^+ \) activity in distal tubules can be estimated. Normally \( Cs^+ \) and \( Rb^+ \) are not present so their high selectivities are not a problem.
NH₄⁺ and H⁺ are both present and have relatively low selectivity ratios. H⁺ concentrations, however, are at least three orders of magnitude lower than potassium so interference by H⁺ can be neglected. NH₄⁺ concentrations are higher and in the tubule lumen may equal potassium concentrations(19). NH₄⁺ would be expected to contribute about 20% to the ion-exchange potential in a solution of NH₄⁺ and K⁺ salts of equal concentration. In the distal lumen, this degree of interference may not be negligible. In tubular cells NH₄⁺ activity is lower and would not be expected to interfere with K⁺ measurements. Selectivity ratios for K⁺ over Na⁺ have been found up to 100:1, but are usually lower for microelectrodes. Because of the high concentrations of Na⁺ present, especially in plasma and luminal fluid, Na⁺ is the most important interfering cation. In plasma, Na⁺ may contribute approximately 10% to a microelectrode measurement of K⁺ activity. When both Na⁺ and K⁺ change, and the changes are not proportional, as is probably the case on impalement of the distal tubule, the variable contribution of Na⁺ to the potential is a source of error that must be considered.

A clear glass surface interacts with water. If an apparently dry glass tube is filled with the liquid ion exchanger, a hydrated channel persists between the organic liquid and the glass surface. In constructing practical microelectrodes, containing the liquid ion-exchanger solution at the tip, it is necessary first to treat the inside of the glass capillary to render it hydrophobic(7,8,11). This has been done by exposing the inner surface to a dilute solution of a silicone in a volatile organic solvent. The silicone compound binds on the glass surface to the charged sites that would otherwise interact with water. Heat curing the silicone drives off water of hydration. Microelectrodes, therefore, have been constructed by pulling tubing into micropipets with tips less than 1 μm. The tip region is filled with silicone solution and baked for 1–2 hrs at 250°. The tip region is then filled with liquid ion-exchanger solution and the stem of the pipet is filled from behind with the reference electrolyte solution—both NaCl and KCl have been used(7,8,13).

| Ion  | \( K_{i/k^+} \) |
|------|----------------|
| Cs⁺  | 0.025          |
| Rb⁺  | 1              |
| K⁺   | 4              |
| NH₄⁺ | 10             |
| H⁺   | 50             |
| Na⁺  | 98, 92, 50, 40–70 |
| Ca²⁺ | 330, 500       |
| Li⁺  | 900            |
| Mg²⁺ | 990            |

* Measurements made with interfering cation at 0.1 M using macro- or microelectrodes constructed with Corning No. 477317 liquid ion exchanger. Values collected from References (13)*, (7)*, and (8)* and from our own measurements.*
Attempts to measure K⁺ activity in the cells and lumen of renal distal tubules face several difficulties that have not been as prominent in previously published studies of mollusk neurons(6,20) or renal proximal tubules(8). The experimental design devised to counter these difficulties is shown schematically in Fig. 2. Starting with the bathing solution over the kidney, the electrode is moved from a solution that has a composition like that of interstitial fluid into the cell and then into the lumen. The K⁺ concentration probably rises and then falls again upon entering the lumen. The Na⁺ concentration is probably lower in the cells than in the lumen. Also along this same route, the membrane potential falls as the peritubular membrane is crossed and rises slightly as the tip enters the lumen. Thus, the change in potential on impaling the distal tubule is a complicated sum of the effects of changes in K⁺, Na⁺ (and probably NH₄⁺) activities and membrane potential. As illustrated in Fig. 2, we decided that, to eliminate some of the confusion, it would be necessary to fabricate double-barrelled micropipet electrodes, one barrel filled with ion-exchanger and the other with an electrolyte solution, here lithium acetate, for simultaneous measurement of the membrane potential. The difference in these potentials is the result of the ionspecific boundary potential at the electrode tip.

An additional problem presented by the tubule bears consideration. The small potential generated at the tip of electrolyte-filled micropipet electrodes is dependent in part on the ionic strength of the outside solution, becoming more negative with decreasing ionic strength. The tip potential also depends on the ionic strength of the solution filling the micropipet as well as the mobilities of the ions involved. In varying concentrations of solutions of the same electrolyte in the pipet, the tip potential becomes increasingly negative as the ratio of outside ionic strength to inside ionic strength decreases. When the ionic strength outside exceeds that inside, the tip potential becomes positive. Distal fluid contains significant amounts of nonelectrolyte solute and may at times be hypotonic. Its ionic strength is less than half that of interstitial fluid. Accurate measurement of distal transepithelial potential differences requires correction for the small change in tip potential that occurs when the tip enters the distal lumen.

**Fig. 2.** Circuit arrangement used in experiments with renal tubule (shown schematically in cross section). Reference electrode is connected to bath fluid by a salt bridge. The double-barrelled micropipet electrode simultaneously measures Em, the membrane potential, and E, the sum of Em and the ion-exchange potential.
Using the circuit in Fig. 2, the procedure was to calibrate each electrode initially in a series of solutions of KCl, NaCl, and mixtures of varying KCl with NaCl constant at 50mM or 150mM. Data from such calibration measurements are shown in Fig. 3. The total circuit potential is plotted against cation activities. The activities are calculated from measured concentrations using activity coefficients estimated from mean ionic activity coefficients using the MacInnes assumption(21,22). The open triangles show the results of measurements with solutions of KCl alone. Line A is fitted by eye to these points and for this electrode has a slope of 58 mV/10-fold change in K⁺ activity. The open circles are the results of measurements in solutions of NaCl alone. The solution containing 155 mM NaCl gives the same potential as a solution of KCl 40 times more dilute. The empirically determined selectivity ratio at this Na concentration is, therefore, 40:1. Line B is fitted by eye to the NaCl data and has a slope of 35 mV/10-fold change in Na activity. Wise et al.(13) and Khuri et al.(8) have also found that measurements in NaCl solutions yield slopes less than theoretically expected for a cation specific electrode. A deviation of this magnitude could be due to the presence of K⁺ at concentrations less than 1 mM, but such contamination of NaCl solutions, if uniform, would not give the straight line we have observed here. The dotted line, C, is calculated from the calibration equation for varying K⁺ activities with a constant background of 155 mM NaCl, and a selectivity ratio of 40:1. The crosses are the results of measurements in such mixed solutions made as a check for this calculation. Finally, the broken line, D, is calculated for varying K⁺ activity against a constant background of 50 mM Na and gives an approximation of the possible range of error in the calibration line produced by unknown changes in Na concentrations when the tubule is punctured. A 5% steeper slope would be expected for in vivo measurements at 38°. The final calibration line falls about midway between Line D and Line A. On this line, in the 5-mM activity region, a 1-mV change in potential corresponds to a 0.2-mM

![Fig. 3. Electrode calibration. Potential established at 25° with solutions of KCl or NaCl alone or mixtures of varying KCl with constant NaCl. Lines designated A–D are explained in text.](image-url)
change in activity. The error that would be introduced by not correcting for the change in Na⁺ activity would be 2.5 mEq/liter in luminal measurements.

Data have also been obtained that permit a calibration of changes in the tip potential of the other barrel, the electrolyte-filled pipet that measures the membrane potential (Fig. 4). Increasingly negative potentials are recorded in increasingly dilute solutions of either NaCl or KCl. The open symbols that scatter about the upper curve are results of measurements with electrodes filled with 3 M KCl and that had tip potentials of 5 mV or less when measured in 155 mM NaCl. The average change in tip potential when the ionic strength is reduced to 60 mM is 4 mV. Larger changes in tip potential were seen with electrodes filled with either NaAc or LiAc as indicated by the data around the lower curve. Here the average change in tip potential when the ionic strength is reduced to 60 mM is 8 mV. As a part of our calibration procedure we measure the change in tip potential of the electrolyte-filled barrel going from a solution similar to interstitial fluid to one like distal tubule fluid. Thus, for each electrode, the characteristics of both the ion-exchanger barrel and the voltage recording barrel are checked before and after in vivo measurements.

Results of measurements made with these electrodes during impalements of the distal tubule of normal rats are shown in Fig. 5. Here the transepithelial potential difference, corrected for change in tip potential, is plotted against the potassium concentration calculated from the ion-exchange potential. The triangles are values obtained with the voltage-recording barrel filled with 3 M KCl. They seem to be falsely high since chemical measurements of distal potassium concentration have not exceeded 25 mEq/liter (23,24). We thought it possible that the ion exchanger was sensing a high K⁺ activity at the electrode tip because of K⁺ leaking from the second barrel. The circles show measurements we then made with the voltage recording barrel filled with 3 M NaAc. These

Fig. 4. Effect of test solution ionic strength on tip potential of electrolyte-filled microelectrode. Test solutions were either KCl or NaCl. Microelectrodes were filled with KCl, NaAc, or LiAc and had tip potentials of less than 5 mV measured in 150 mM NaCl. Points are averages of measurements. Vertical lines indicate ± 1 SEM.
values generally fall into the range of concentrations previously measured in collected samples of tubule fluid. More recently we have used electrodes filled with 3 M LiAc solution in the voltage recording barrel on the assumption that the Li+ should interfere only a tenth as much as Na+ (Table 1). In practice values with the LiAc electrodes were found to be similar to the ones shown for NaAc electrodes. It was concluded that neither Na+ nor Li+ in the second barrel interferes appreciably with the ion-exchanger measurements. Values in the lower range of PD were obtained from impalements of the more proximal part of the distal convolution and those in the higher range from the late portion. The electrode measurements show the increase in potassium concentration along the length of the distal tubule that has been found in earlier studies using chemical measurements(23,24). Both the values for distal P.D. and K concentrations are from recordings that were stable for approximately 1 min. We do not yet have what we consider an adequate independent means of localizing the tip as either in the cell or lumen. From our past experience(25) one would expect that the majority of these measurements represent luminal potassium concentrations. Very occasionally K+ concentrations ranging from 100 to 300 mEq/liter have been observed using Li+-filled electrodes. Although these results are only preliminary, the method appears to be a promising approach to measuring K+ activities in renal tubule cells.

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![Graph](image-url)  
**Fig. 5.** Relation between simultaneously measured distal electrical potential difference and potassium concentration. Points are individual measurements.
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