Electrophoretic Method of Ion Injection in Single Kidney Cells*

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Received for publication 19 November 1971

Electrophysiological studies of the electrochemical profile across the renal epithelium have contributed to our understanding of the mechanism responsible for sodium reabsorption. In particular such studies have defined the electrical potential step across the luminal and peritubular cell membrane and have made it possible to locate a mechanism for active Na extrusion at the peritubular cell boundary(1). Few techniques have been available to study transport phenomena at the single cell level. One way to obtain information at this level is to conduct studies on tissue slices. Alterations of intracellular ion content and cell potential difference in kidney slices exposed to a variety of soaking procedures permit an analysis of ion fluxes across single cell membranes(2). Slice techniques, however, lead to the disruption of the tubular geometry of the renal epithelium and of the normal asymmetrical behavior of kidney cells with respect to transport.

Ideally, intracellular ion concentrations should be modified by means of a technique maintaining the integrity of the tubular epithelium and allowing for the assessment of the sodium pump at the single cellular level. To this end, we developed an intracellular electrophoretic ion injection technique applicable to Necturus proximal tubule cells. Similar methods have been used extensively in excitable tissues(3).

In the in vivo or doubly-perfused kidney of Necturus, it is possible to impale single proximal tubule cells by means of a double-barreled microelectrode as indicated in Fig. 1. The peritubular membrane potential difference can be recorded as well as its alteration after cation injection in the same cell. One barrel of the electrode is filled with 3 M KCl and measures the potential difference between the cell interior and the surface of the kidney. The other barrel is used for current injection and contains concentrated solutions of potassium acetate,

* This investigation was supported by PHS Research Grant 1-RO1-AM-13844 from the National Institutes of Arthritis and Metabolic Diseases.
rubidium acetate, sodium acetate, lithium acetate, or tetraethylammonium-chloride. Cations are ejected from the tip of the electrode in a single proximal tubule cell by application of depolarizing current from a constant current source for different periods of time. For instance, a current barrel filled with sodium acetate would eject sodium ions from the tip of the electrode, increasing the intracellular sodium concentration. However, current also flows through the peritubular membrane and causes an ion shift at that border, i.e., potassium ions leave the cell to the extracellular fluid. Furthermore, since the applied current can be carried both by cation and anion movement the efficiency of a cation injection can be improved by reducing the fraction of the anionic current. This can be achieved by decreasing the concentration of extracellular or intracellular ions of high mobility. In our experiments chloride is substituted for in the extracellular fluid by a larger anion such as isethionate. Consequently, the expected intracellular ion changes after application of a depolarizing current across the sodium-filled barrel are a rise in intracellular sodium and a fall in intracellular potassium concentration.

Figure 2 illustrates the responses of the peritubular membrane potential to sodium injection. Injections were repeated three times in the same cell by applying current of 58 nA for time periods of 1 min, 10 sec, and 2 min, respectively. Subsequent to each injection, a depolarization of the peritubular membrane potential was noted, the amplitude increasing with a longer time of current flow. The membrane potential repolarizes with time to the preinjection level. The time course of recovery was found to consist of two different exponential terms. The difference in potential from the preinjection level is plotted in the

**Figure 1.** Method for the electrophoretic injection of cations in single proximal tubule cells. The left barrel of the microelectrode contains a concentrated solution of a salt of the cation to be injected and is connected to a constant current source. The right barrel is filled with 3 M KCl and records the peritubular membrane potential.
Fig. 2. Top: Example of three injections of Na, with a constant current of 58 nA, during different time intervals as indicated by the vertical lines. Transient changes in peritubular membrane potential monitored by the second barrel of the microelectrode. Bottom: Change in membrane potential compared to the preinjection level plotted as the logarithm against time. Analysis in two exponential functions. For explanation see text.

lower part of Fig. 2. The total potential change immediately after cessation of the current application at time zero is \( \Delta V_o \). When the curve is decomposed into the sum of two exponential functions, the first fast component has an amplitude \( \Delta V_o^1 \), and a half-time of repolarization \( t_{1/2}^1 \) while the slow component has an initial amplitude \( \Delta V_o^{11} \), with a half-time of repolarization \( t_{1/2}^{11} \).

The numerical values of this analysis as applied to sodium injection are given in Table 1. It appears that the slow component has a half-time one order of magnitude larger than the fast component. To determine which of these components is related to active sodium extrusion, additional experiments were performed. Essentially, these studies consisted of a comparison of the electrogenic effects of injection of ions other than Na in the cell interior, changing the ion composition of the peritubular fluid, or influencing the active transport mechanism by means of cooling, or by metabolic inhibition with ouabain or ethacrynic acid. The available evidence indicates that the first component is due to an extracellular phenomenon. It would be the consequence of potassium accumulation in an extracellular space close to the peritubular cell border. It is likely that this subcompartment of the extracellular fluid exchanges relatively slowly with the peritubular fluid space. Repolarization occurs by removal of potassium from this area of restricted diffusion. Regarding the nature of the second component, it appears to result from an intracellular depletion of potassium ions
TABLE 1

| | Intracellular Na⁺ Injection (15 sec) Extracellular Ringer Without Cl⁻ | |
|---|---|---|
| ΔV₀ (10⁻⁸V) | +13.5 ± 0.56 (57) | |
| ΔVᵢ (10⁻⁸V) | + 6.1 ± 0.42 (43) | |
| tᵢ (min) | 2.3 ± 0.24 (43) | |
| ΔVᵢ (10⁻⁸V) | + 7.6 ± 0.42 (43) | |
| tᵢ (min) | 18.7 ± 1.17 (43) | |

*Values are means ± SE followed by number of observations in parentheses.

and an enrichments in sodium ions. The recovery of this component is accounted for in terms of the activity of a sodium–potassium exchange pump, restoring the intracellular potassium content.

Under special experimental conditions a response can also be demonstrated that is probably the manifestation of an electrogenic sodium pump mechanism in proximal tubule cells. This is a mechanism by which the extrusion of Na from the cell into the peritubular fluid directly contributes to the generation of a negative membrane potential. When the kidney is perfused with potassium-free Ringer for about 3 hr, the peritubular membrane potential declines because of a washout of intracellular potassium. Under these conditions the injection of Na, but not Li ions induces a transient hyperpolarization instead of a depolarization. Cooling reduces the magnitude of the response, and ethacrynic acid abolishes the hyperpolarizing transient. This and other evidence supports the thesis that the observed hyperpolarization is due to the activity of an electrogenic pump component.

In conclusion, the electrophoretic ion-injection technique can be successfully applied to single tubule cells. It provides a convenient way for altering the intracellular ion composition in a reversible fashion. The analysis of the transient membrane potential changes after different ion injections offers a powerful tool for the assessment of the passive ion permeability of the cell membranes. Most importantly, it allows for a characterization of the properties of the active sodium extrusion mechanism at the single cell level.

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