Single-Channel Techniques Applied to Kidney Proximal Tubule Apical Membranes

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New electrophysiological methods, related to single-channel recording techniques that enable investigation of the molecular components participating in transport mechanisms, have been developed in the last decade. Applications of these methods to renal physiology indicate possible implications for present concepts of proximal reabsorption.

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Human kidney proximal convoluted tubules transport ~120 liters of water per day, together with 1.5 kg of NaCl from the urine-forming compartment, the lumen, to the extracellular space. This transport process costs the kidney 80% of its oxygen consumption. The kidney itself expends 7–8% of the total body oxygen consumption, with 20% of the cardiac output being diverted to it. The importance of the proximal convoluted tubule in regulation of blood electrolytes instigated a search for methods to gain access to the apical and basal membranes of epithelial cells, the site of the transport process.

Ideally, any study of transport mechanisms should include two approaches: microscopic and macroscopic. The first aims at identifying the transport process. The second approaches: microscopic and macroscopic, The first aims at identifying the transport process. The importance of the proximal convoluted tubule is a matter for debate. However, since no passive sodium conductance was found through the luminal membrane, sodium-permeable channels were deleted from this model, albeit only temporarily. These conclusions were based on macroscopic measurements.

The need for support of this model with microscopic measurements led electrophysiologists to turn to new techniques. For these techniques to fully uncover the molecular nature of the transport mechanism in the membrane, they have to enable recordings of transport of solutes in channels, pumps, and carriers at the single molecule resolution, and they must overcome the restrictions imposed by the highly complex geometry of the proximal convoluted tubule. No such techniques are available at the single carrier molecule level. The contribution of this transcellular sodium transport to the overall transepithelial reabsorption process is a matter for debate.

FIGURE 1. Schematic representation of various components that play a role in water and salt absorption in proximal convoluted tubule. Except for Na channel, all components are based on macroscopic measurements. See text for further description.
level for noncharged solutes; therefore their detection is still confined to macroscopic fluxes at present. Electrogenic transport, on the other hand, can be monitored directly at the single-channel level with a lower detection limit of ~1 pS. In contrast to the turnover numbers of ionic pumps and carriers, 10^9 and 10^10, respectively, ionic channels transport ions at a rate of 10^8 ions/s, a current detectable by standard electronic amplifiers.

A decade ago, Hamil et al. (9) introduced the patch-clamp technique by which a single-channel ionic current can be recorded (Fig. 2). This technique cleared the way to a new era in electrophysiology. It is based on the ability to seal a glass pipette onto a patch of membrane, establish a desired potential difference across that patch, and eliminate current flow from the pipette to the external medium. A precondition for utilization of this technique is that the patch of membrane to be clamped must be accessible. This is not immediately obvious in the luminal membrane of the proximal convoluted tubule. The membrane here is microvillous and secluded within the convoluted lumen. In the following sections we introduce several ways to circumvent these difficulties, applicable to epithelial tissues in general and to luminal membranes in particular.

**Bilayer techniques**

For more than a decade methods have existed to isolate relatively pure apical membrane fractions from the proximal convoluted tubule. Membranes are precipitated by either calcium or magnesium ions, yielding an enrichment of 10- to 100-fold with respect to brush-border marker enzymes (2) such as alkaline phosphatase. While these vesicles are used largely to record macroscopic fluxes, two methods allow their use for microscopic single ionic channel current recordings, “tip dipping” and reconstitution into planar bilayers.

**Tip dipping: incorporation of channels from vesicular preparations into a lipid bilayer formed at the tip of a patch-clamp pipette.** This method, introduced in its final version by Coronado and Latore (3), is based on the tendency of a liposome suspension to form a monolayer at the water-air interface. Minutes after addition of vesicles to an aqueous solution, vesicles that ascend and reach the surface unfold and form a monolayer of lipids with their hydrophobic tails facing up. If these vesicles contain membrane proteins, channels in our case, these are inlaid in the monolayer. Passing a glass capillary twice through the monolayer will result in formation of a bilayer at the tip of the pipette. The pipette, serving as a patch-clamp electrode, can be moved to adjacent chambers with different experimental solutions to identify and characterize the channels entrapped in the tip. Using this method, we were able to demonstrate the existence of cation-permeable channels in the apical membrane of the proximal convoluted tubule of the rat (11). These channels, being equally permeable to sodium and potassium, function as sodium channels under physiological conditions where the electrochemical gradient for sodium is 10-fold higher than the potassium gradient.

The tip-dip method has its limitations. 1) We do not know how physiological functions of the protein are affected by floating in the water-air interface. 2) Proteins embedded in artificial membranes may function differently. 3) Channel proteins may be altered through the vesicle preparation procedure. 4) Reconstitution of a channel deprives it of cellular modulatory systems. 5) The purity of the preparation is estimated at 95%. One can imagine a situation in which the concentration of ionic channels in the contaminant is much higher, masking the activity of the investigated membrane. On the other hand, isolation of the channel protein from cellular control and temporal resolution of its gating activity are maximized in the tip-dip method.

**Big bilayers: incorporation of channels from a vesicular preparation into a planar bilayer formed across a chamber partition.** The incorporation of proteins into a planar bilayer is a well-established process. The method has been used to study properties of amiloride-sensitive channels from kidney cell lines (13). Conditions are set for vesicles in the cis side of the partition to fuse with a lipid bilayer separating it from the trans chamber. Usually these conditions include the addition of calcium ions and formation of an osmotic gradient to induce movement of water to the cis side, thus causing the vesicles to swell and fuse with the bilayer. Using the planar bilayer system, we demonstrated and characterized cation-selective channels derived from the proximal convoluted tubule, similar to channels recorded in the tip-dip setup described above (11).

The only limitation of this method, compared with tip dipping, is that the temporal resolution here is much lower due to the very high (>300 pF) capacitance of the bilayer. On the other hand, recordings with this technique are more stable and the channel faces aqueous solutions from both sides. When channel-gating activity under investigation is slow (in the range of 30–50 Hz), this method is the technique of choice.
Most of the shortcomings of the tip-dip version of channel monitoring apply also to planar bilayers. If one is to make conclusions about channel activity in the native membrane, experiments with vesicular preparations may be insufficient.

**Micromanipulating the tubule**

Gogelein and Greger (7) developed an elegant method for recording single-channel activity from the luminal membrane of the renal proximal tubule. Tubular segments were cannulated and perfused from the proximal side, with the distal tube free on the bottom of a perfusion chamber. It is possible to insert a patch pipette into the lumen at the open end of the tubule and to record single channels. Using this technique, Gogelein and Greger were the first to demonstrate the existence of sodium-permeable channels in the luminal membrane of the pars recta (the straight part of the proximal tubule). The channel conductance is 12 pS with a high selectivity for sodium over potassium ions. In addition, this channel is sensitive to amiloride, which shortens the mean open time.

This technique is the most direct demonstration of naturally occurring conductances in the intact tubule. It is not at all obvious that this method will enable single-channel recordings from the luminal membrane of the convoluted section where most of the activity associated with the luminal membrane of the convoluted tubule takes place. Nonetheless, reabsorption in the proximal convoluted tubule. We believe that the apical sodium channel may play an important role in proximal convoluted tubule reabsorption by controlling the transcellular sodium ion concentration. As seen in the scheme (Fig. 1) recording of single-channel activity with the entire cellular machinery left intact. Most important are the factors and processes that are involved in membrane conductance modulation. Patching cells in culture is more convenient than the Gogelein and Greger (7) method but manipulation of the cells for culturing and the risk of alterations of intrinsic characteristics with consecutive cell divisions must be taken into account.

**Which technique should one use and when?**

The above are some of the techniques available today to study the conductance of proximal convoluted tubule membranes, each with its potential advantages and pitfalls. The method of choice depends on the specific question addressed. In our experience, questions concerning the existence of a particular conductance in a given luminal membrane are best answered with the Gogelein and Greger (7) method or in tissue culture. When the questions concern biophysical characteristics of a certain channel, one should choose a vesicular method with preference to the tip dip only when the temporal resolution needed is higher than that offered by the planar bilayer system.

Are we reading a book through an electron microscope?

By no means can single-channel data alone give a comprehensive view of the way a cell or tissue behaves as a whole. Yet an ionic current detected at the single-channel level may have profound effects on cellular function and still be below threshold for detection macroscopically. It is still widely accepted, on the basis of the elegant macroscopic data provided by Fromter (4) and others, that no sodium channels exist in the luminal membrane of the proximal convoluted tubule. All the transcellular transport of sodium is attributed to coupled mechanisms, the most popular being the Na-H exchanger. On the other hand, sodium conductances were clearly demonstrated by each of the above-mentioned single-channel recording techniques. Gogelein and Greger (8) suggested that "the sodium channel mechanism plays only a minor role for total sodium transport" in the proximal convoluted tubule. We believe that the apical sodium channel may play an important role in proximal convoluted tubule reabsorption by controlling the transcellular sodium ion concentration. The concentration changes due to influx of ions through a small number of channels, on the order of five channels per cell, are sufficient to serve as a modulator. In various tissues, calcium-dependent potassium channels, sodium-dependent potassium channels, chloride-dependent G proteins, calcium-dependent enzymes, and sodium-dependent amino acid fluxes are all modulated by ion concentrations altered via currents through a minimal number of channels. These currents, which are undetectable by macroscopic methods, may have profound consequences for the cell's function. Thus the claim that the apical membrane of the convoluted proximal tubule is devoid of a

**Primary cultures of isolated proximal convoluted tubule**

Epithelial cells in culture form a monolayer with free access to the apical (luminal) membrane. This significantly simplifies electrophysiological studies of ionic channels present in apical cell membranes. Electron microscopy is usually carried out to verify the microvillous nature of the membrane at the apical side and the existence of tight junctions between cells of the monolayer. There are several techniques to obtain epithelial cell monolayers. Merot et al. (12), the first to document single ionic channel activity of proximal convoluted tubule cells in culture, chose to culture defined segments obtained by microdissection. We use Percoll gradients to purify segments of the proximal convoluted tubule as a source of cells for culturing (11). The latter offers a much higher yield at the price of ~5% contamination from other segments of the nephron.

Both Merot et al. (12) and Marom et al. (11) recorded cationic channel activity in the apical membrane of proximal convoluted tubules in culture. These cationic channels are comparable to ones we found previously in the vesicular preparation described above. The use of tissue culture enables recording of single-channel activity with the entire cellular machinery left intact. Most important are the factors and processes that are involved in membrane conductance modulation. Patching cells in culture is more convenient than the Gogelein and Greger (7) method but manipulation of the cells for culturing and the risk of alterations of intrinsic characteristics with consecutive cell divisions must be taken into account.
"meaningful" sodium conductance, based on macroscopic data, must be reconsidered. Single-channel techniques reveal covert sodium channels in these membranes, and their contribution to transport mechanisms, even if indirect, may be of paramount importance. A similar picture seems to be emerging with respect to chloride channels. We can hope that ultimately the exact mechanisms of water and solute transport in the proximal convoluted tubule will be understood by combining single-channel techniques, described above, with macroscopic approaches.

References


In Water Deprivation, Osmolality Becomes an Important Determinant of Aldosterone Secretion

Edward G. Schneider

The adrenal gland secretion of aldosterone is closely linked to the volume and composition of the plasma compartment via the renin-angiotensin II system. In contrast, alterations in the plasma concentration of potassium and in plasma osmolality significantly alter aldosterone secretion by a direct action on the adrenal gland.

Introduction

Aldosterone, a major mineralocorticoid hormone secreted by the glo- merulosa cells of the adrenal gland, stimulates the reabsorption of so- dium and the secretion of potassium and hydrogen ions by the distal nephron segments of the kidney. Hypo- and hypersecretion of aldoste- rone are associated with marked disturbances in the homeostatic regu- lation of sodium balance, leading to disturbances in blood pressure regulation and alterations in the size of the extracellular compartment. Disturbances in both potassium and acid base balance also accompany primary defects in the secretion of aldosterone. Thus the maintenance of both the size and composition of the extracellular compartment is, in part, determined by the ability of the adrenal gland to control the secretion of aldosterone.

Most physiologists recognize that both the activity of the renin-angio- tensin system and the plasma concent- ration of potassium are impor- tant regulators of the secretion of aldosterone by the adrenal glomer- ulosa cells. In fact, these two factors are considered to account for the control of aldosterone secretion un- der most physiological situations. However, studies conducted in a number of laboratories over the past decade have shown that plasma al- dosterone levels cannot be ac- counted for by the concurrent plasma activity of the renin-angio- tensin system or by the plasma concent- ration of potassium in a variety of situations in which the plasma sodium concentration is either higher or lower than normal (3, 10).