A Discussion of *Ambystoma* Kidney Tubule Ion Channels, Transporters, and pH Regulation

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This paper explores the role and biophysical expression of the equivalent electrical circuit model as it applies to ionic conductances across the paracellular shunt, apical membrane, and basolateral membrane of the *Ambystoma* renal proximal tubule. Information about such conductances may be experimentally determined through transepithelial voltage and intracellular voltage measurements.

The equivalent electrical circuit model has been applied extensively by investigators to define ion channels and transport mechanisms in the salamander proximal tubule. A comprehensive discussion of all known ionic conductance and transport pathways as well as pH-regulatory functions of contributory symports/antiports is examined in the *Ambystoma* proximal tubule. This paper explores renal physiological principles and serves as a companion to: Bock JF, Boulpaep EL: Bicarbonate transport mechanisms in the *Ambystoma* kidney proximal tubule: Transepithelial potential measurements. Yale J Biol Med 63:529–547, 1990.

INTRODUCTION

Prior to 1981, intracellular and transepithelial voltage measurements on the salamander proximal tubule preparation were limited to *in vivo* perfusion techniques; however, with the advent of an *in vitro* micropertusion system, precise manipulation of luminal and/or bath ionic compositions became feasible [1]. This technique, in consonance with the *in vivo* perfusion method, permits examination of individual membrane resistance, ion conductance, and potential, using equivalent electrical circuit analysis [2]. Current models of ion channels and transport systems in the *Ambystoma* proximal tubule are expanded with the application of ion-selective electrodes and, more recently, patch-clamp techniques.

EQUIVALENT ELECTRICAL CIRCUIT ANALYSIS

Morphologically, the proximal tubule cell epithelium is composed of three membrane boundaries: the apical, basolateral, and lateral intercellular barriers. The equivalent electrical circuit model represents a framework for describing one or more ionic diffusional barriers where each separate boundary constitutes a potential resistance to ionic current. Schematically, \( R_1 \) represents the basolateral, \( R_2 \) embodies the apical, and \( R_3 \) describes the paracellular membrane resistances (see Fig. 1).

Hence, a single-layer epithelium such as the *Ambystoma* proximal tubule is

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**Abbreviations:** CHC: alpha-cyano-4-hydroxycinnamate  \( V_3 \): transepithelial potential difference

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portrayed as two resistors in series ($R_1$ and $R_2$) with one resistor in parallel ($R_3$) (see [3] for a review). At each diffusional barrier, a corresponding equivalent electromotive force represents the dissipative ionic processes (i.e., $E_1$, $E_2$, and $E_3$; see Fig. 1). The electromotive force for a permeant ion across a particular diffusional barrier is defined by its corresponding Nernst equilibrium potential ($E_j$), ionic current ($i_j$), and ionic resistance ($R_j$). Therefore, measurements of $V_3$, the transepithelial potential difference, are a function of $R_1$, $R_2$, and $R_3$, as well as $E_1$, $E_2$, and $E_3$. Mathematically, this is expressed as [2]:

$$V_3 = \frac{(E_1 + E_2)R_3 + E_3(R_1 + R_2)}{(R_1 + R_2 + R_3)}$$

This equation applies exclusively to transepithelial potentials produced by dissipative ionic forces. The equivalent electrical circuit model is, of course, incomplete in the presence of apical and/or basolateral active ionic transporters. In the *Ambystoma* proximal tubule, dissipative forces alone cannot account for experimentally determined transepithelial potential differences under the conditions of symmetrical apical and basolateral solutions (i.e., see [4]: evidence for a basolateral rheogenic Na$^+$-K$^+$ antiport). Two rheogenic current sources are added to Fig. 1 where $i'_h$ refers to active basolateral current and $i'_a$ defines active apical current sources (Fig. 2). The current sources at either membrane may be caused by positive or negative ionic currents. Any disparity between the two current sources is defined by $i_c$ (where $i_c = i'_h + i'_a$). Combining the diffusional and rheogenic sources of transepithelial potential difference
results in the expression [2]:

\[
V_3 = \frac{[(E_1 + E_2)R_3 + E_3(R_1 + R_2)]}{(R_1 + R_2 + R_3)} - \\
\text{diffusional terms}
\]

\[
i_1^p[R_1R_3/(R_1 + R_2 + R_3)] - i_2^p[R_2R_3/(R_1 + R_2 + R_3)]
\]

rheogenic terms

DIFFUSIONAL AND IONIC COMPONENTS DESCRIBED IN THE AMBYSTOMA PROXIMAL TUBULE

The equivalent electrical circuit model may be applied to the Ambystoma proximal tubule. Figure 3 depicts ionic channels and various transporters identified along the apical and basolateral membranes. A highly Cl\textsuperscript− selective paracellular current has also been identified in Ambystoma proximal tubule [4]. To facilitate discussion of rheogenic sources of apical and basolateral current, it is useful to divide defined ionic channels and transporters into electroneutral and rheogenic components.

Patch-clamp experiments have identified potassium channels along the basolateral and apical membranes where the number of basolateral K\textsuperscript+ channels exceeds [5] those on the apical surface [6]. Under physiological circumstances, E\textsubscript{K\textsuperscript+} (the electromotive force for K\textsuperscript+) favors bilateral potassium extrusion. If the intracellular pH increases, K\textsuperscript+ channel conductance may increase [7,8], producing a change in E\textsubscript{K\textsuperscript+}, since the number of K\textsuperscript+ channels is greatest along the basolateral membrane; this condition is a potential source of changes in V\textsubscript{3} in altered intracellular pH states. Chloride channels have recently been identified within the apical and basolateral membranes [9]. Under
physiological conditions, $E_{Cl^-}$ favors bilateral chloride extrusion through $Cl^-$ channels. Na$^+$ channels have been found along the apical membrane by patch-clamp analysis [6] and by in vitro perfusion [10]. $E_{Na^-}$ favors Na$^+$ absorption from lumen to cell interior in the normal physiological state.

**PRIMARY-, SECONDARY-, AND TERTIARY-ACTIVE TRANSPORT SYSTEMS DESCRIBED IN THE AMBYSTOMA PROXIMAL TUBULE**

Electroneutral transporters include the apical Na$^+$/lactate and basolateral H$^+$/lactate symporters [11], the Na$^+$-H$^+$ antiporters located on both membranes [7], and the basolateral Na$^+$-dependent HCO$_3^-$-Cl$^-$ antiporter shown as Na(2HCO$_3^-$)-Cl antiporter [7,9]. The Na$^+$-H$^+$ antiporters are electroneutral and are oriented in opposite directions at the two cell membranes (see Fig. 3). The proposed electroneutral Na$^+$/$Cl^-$ symporter and Cl$^-$-Anion$^-$ antiporter have not been identified along the apical membrane; however, Cl$^-$-Anion$^-$ antiporter has been localized to the basolateral surface [9,10]. Other speculated sources of apical Na uptake, namely the proposed Na$^+$/2Cl$^-$/K$^+$ co-transporter, have recently been disproven [9].

Therefore, potential sources of rheogenic current are relatively few in number in the Ambystoma proximal tubule. These transporters are either primary-, secondary-, or tertiary-active mechanisms (see [12] for review). For example, the major source of positive $i'_p$ current is the influx of Na$^+$ current with co-transport of organic substrates; this is by definition a secondary-active co-transporter, as Na$^+$ current depends upon the presence of substrate [13], whereas the 3Na$^+$/2K$^+$ antiporter is a primary-active
system, as the antiport depends upon ATPase [12]. The basolateral H⁺/Lac⁻ symporter is an example of a tertiary-active system where Na⁺ entry across the apical membrane is facilitated by the presence of lactate (a secondary-active mechanism); H⁺ efflux across the basolateral membrane depends upon the intracellular accumulation of lactate: hence this is a tertiary-active system (see [12]).

Sources of basolateral electrogenic current (i_b) include the primary-active 3Na⁺-2K⁺ ATPase [2] and the secondary-active Na⁺/(HCO₃)₉ symporter [14]. The former mechanism represents a source of positive current iₐ, whereas the latter encompasses a source of negative basolateral current iₐ.

**pH Regulation in the Ambystoma Proximal Tubule**

Intracellular pH is carefully balanced by two interacting homeostatic processes: (1) transmembrane fluxes of H⁺ ions (or equivalent weak acid form) and (2) transmembrane movement of HCO₃⁻ or related OH⁻ or weak base species (see [15] for an excellent review of intracellular pH regulatory mechanisms in epithelia). Within the Ambystoma proximal tubule cell, a variety of acid/base absorptive and secretory mechanisms have been experimentally described (see Fig. 2 of Bock JF, Boulpaep EL: Bicarbonate transport mechanisms in the Ambystoma kidney proximal tubule: Trans-epithelial potential measurements. Yale J Biol Med 63:529-547, 1990, for a current model of intracellular pH regulation in Ambystoma).

As noted in this figure, the Ambystoma proximal tubule epithelium is highly permeable to CO₂ and readily diffuses across the cell membrane down its electrochemical gradient. Intracellularly, CO₂ is hydrated to carbonic acid (H₂CO₃) in a reversible chemical reaction, which then dissociates into H⁺ and HCO₃⁻. Furthermore, H₂CO₃ also diffuses readily across the proximal tubule membranes following its chemical gradient. Both diffusional pathways augment the intracellular concentrations of H⁺ and HCO₃⁻. If an acid-equivalent or base-equivalent transporter exists on either or both of the apical or basolateral membranes, such mechanisms may or may not produce a perturbation of intracellular pH, depending upon transport stoichiometry, physiological orientation, and intracellular pH buffering capacities. Such processes may be pH-dependent where they are activated or inactivated in circumstances of altered intracellular pH.

Electroneutral H⁺ transporters include apical and basolateral membrane Na⁺-H⁺ antiport and the Na⁺-Lac⁻ and H⁺-Lac⁻ co-transport systems. Evidence for a mammalian proximal tubule Na⁺-H⁺ antiport is well documented [16]. Conclusive evidence for the Na⁺-H⁺ antiport is described in rat [15] and rabbit proximal tubule membrane vesicle studies [16]. In Ambystoma in vitro perfused tubules, Boron and Boulpaep [7] demonstrated that intracellular pH recovery occurs spontaneously if cells are initially acid-loaded and maintained in symmetrical, nominally free HCO₃⁻ Ringer. This pHᵢ recovery is also accompanied by a transient increase in intracellular Na⁺ activity (a Naᵢ) and is blocked by symmetrical Na⁺ deletion and by the addition of bath and/or luminal amiloride (a blocking agent which is relatively specific for Na⁺/H⁺ antiport systems) [7]. In the presence of luminal and bath HCO₃⁻, intracellular pH is lower than in the symmetrical HCO₃⁻-free and CO₂-free situation. When HCO₃⁻ is administered on both sides of the epithelium, electrochemical gradients for H₂CO₃ and CO₂ favor intracellular entry of both species, which tips the chemical equilibrium toward H₂CO₃ dissociation. The rheogenic basolateral Na⁺/(HCO₃)₉ transporter promotes HCO₃⁻ exit, which imposes a net acid load upon
the intracellular milieu [14]. Hence, in the presence of HCO$_3^-$, the Na$^+$-H$^+$ antiport system may serve as a mechanism for reducing the rate of acid loading.

The electroneutral Na$^+$/Lac$^-$ and H$^+$/Lac$^-$ co-transporters have recently been elucidated with in vitro perfusion studies in Ambystoma [11]. In these experiments, symmetrical exposure to a luminal and bath lactate source produced a detectable increase in intracellular pH (on the order of 0.2 pH unit). If lactate and Na$^+$ are exclusively applied to the lumen, intracellular pH rises without generating a change in transepithelial potential; this information suggests that the apical transporter is an electroneutral process. In the circumstance of a lactate source in the bath only, intracellular pH falls approximately 0.08 pH unit; this effect is not altered by removal of bath Na$^+$; however, it is reversely decreased by bath CHC (alpha-cyano-4-hydroxyxinnamate), a H$^+$/dicarboxylate transport inhibitor. From these findings, it was concluded that the net effect of the operation of apical Na$^+$/Lac$^-$ and basolateral H$^+$/Lac$^-$ co-transporters is a net transepithelial efflux of lactate, basolateral membrane H$^+$ extrusion, and a rise in intracellular pH.

As a pH regulator, the lactate co-transport system is quite important in the circumstance of bilateral HCO$_3^-$ removal where CO$_2$ and H$_2$CO$_3$ diffuse out of the intracellular compartment, thereby increasing intracellular pH. In the presence of a 3.6 mM luminal and bath lactate source, intracellular pH increases by approximately 0.2 pH unit. Therefore, in the presence of a lactate source and absence of symmetrical HCO$_3^-$, the Na$^+$/Lac$^-$ and H$^+$/Lac$^-$ co-transporters represent a major intracellular alkalinization system. As noted previously, when lactate is unavailable to the tubule, the Na$^+$-H$^+$ exchanger acts as the major intracellular alkalinization mechanism and thereby sets a limit on how far intracellular pH can fall.

Related electroneutral pH regulating mechanisms which have been described in the basolateral membrane of Ambystoma include the Cl$^-$-Anion$^-$ and [Na$^+$/HCO$_3^-$ and Cl$^-$/H$^+$] exchanger. The former system appears to be sensitive to DIDS, a disulfonic stilbene derivative which selectively blocks Cl$^-$-HCO$_3^-$ and Cl$^-$-Anion$^-$ antiports, whereas the latter depends upon the presence of a basolateral Na$^+$ source [9]. These experiments are somewhat limited, as intracellular pH was not monitored; therefore, the specific role of these transporters on intracellular pH regulation remains speculative.

In the Ambystoma proximal tubule, an experimentally identified rheogenic, pH-regulating transporter is the Na$^+$/HCO$_3^-$ basolateral co-transporter [14]. If basolateral pH is reduced from 7.5 to 6.8 by decreasing bath HCO$_3^-$ concentration, intracellular pH rapidly falls (approximately 0.35 pH unit), and the basolateral membrane is transiently depolarized. This maneuver also produces a fall in intracellular Na$^+$ activity; the effect is reversible. Alternatively, deleting bath Na$^+$ in the presence of a HCO$_3^-$ bath yields a rapid fall in intracellular pH and a transient basolateral depolarization. Both experimental maneuvers are blocked by SITS, unaffected by Cl$^-$ removal, and fail to demonstrate appreciable changes in intracellular Cl$^-$ activity. Indeed, these experiments provide solid evidence for a basolateral Na/O$_3$ co-transporter with a ratio of HCO$_3^-$ to Na$^+$ flux greater than unity. Therefore, the number of transported HCO$_3^-$ molecules "n" is regarded as >1.

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