Biochimica et Biophysica Acta, 555 (1979) 519—523

BBA 78471

CURRENT-VOLTAGE RELATIONSHIP OF THE BASOLATERAL MEMBRANE OF A TIGHT EPITHELIUM

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(Received January 25th, 1979)

Key words: Current-voltage relationship; Basolateral membrane; Nystatin; (Rabbit colon)

Summary

The polyene antibiotic nystatin is used to reduce selectively to zero the apical membrane resistance of the rabbit descending colon, allowing the measurement of the current-voltage curve of the basolateral membrane. The $I—V$ relationship is described by the Goldman-Hodgkin-Katz equations allowing calculation of P_{Na}/P_K, P_{Cl}/P_K and P_K for the basolateral membrane. Cs^+ is found to block inward current (serosa → mucosa) in a manner similar to that found in excitable membranes.

Introduction

Recently Fuchs et al. [1] demonstrated that the current-voltage (I-V) relationship of the amiloride-sensitive Na^+ transport system at the apical membranes of frog skin can be accounted for by a model that assumes constant-field rectification. The present communication asks the following questions. Can the permeability equations of Goldman [2] and Hodgkin and Katz [3] be used to model the I-V relationship of the basolateral membrane of the amiloride-sensitive Na^+-transporting epithelium, rabbit descending colon, and are other characteristics of the basolateral I-V relationship similar to those of other preparations?

Methods

Rabbit descending colon was mounted between modified Ussing chambers [4] according to the technique of Frizzell et al. [5]. In order to perform I-V

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measurements on only the basolateral membrane of the colon we had to (1) correct for series resistance; (2) reduce the apical membrane resistance to zero; (3) measure and correct for the tight junction resistance, and (4) mimic intracellular ion composition. We corrected for series resistance by measuring either the voltage response 20 μs after the application of a square-current pulse or the peak current response to a square-voltage step. In the first case a very distinctive resistive step was discernible since the time constant of the membrane was of the order of 2 ms. Both measures yielded the same value for series resistance (approx. 30 $\Omega \cdot cm^2$). Apical membrane resistance was selectively reduced to an immeasurably small value by application of the polyene antibiotic nystatin (120 units/ml) to the mucosal solution [6,7]. The method of Lewis et al. [6] was used to calculate and correct for the influence of junctional resistance on the *I-V* curve. We considered the possibility that the junctional resistance might be voltage dependent. However, since the transepithelial *I-V* relationship in the absence of nystatin was linear, we felt that it was simplest to assume a constant junctional resistance at all voltages tested.

Intracellular ion content was mimicked using the intracellular ion activity measurements of Wills et al. [7]. In all the experiments the mucosal solution was always a K_2SO_4/saline [6] (in mmol: 58.5 K_2SO_4, 25 $KHCO_3$, 10 calcium methanesulfonate, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 11.1 glucose, 80 sucrose) and, except for the Cs^+ experiments, the serosal solution was always a NaCl/saline [8] (in mmol: 111.2 NaCl, 25 $NaHCO_3$, 5.8 KCl, 2.0 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 11.1 glucose). All solutions were pH 7.4 and bubbled with 5% CO_2/95% O_2. The basolateral membrane was clamped for 100 ms at predetermined voltage steps on either side of the resting potential. Each clamp potential was repeated four times and the mean current and clamp voltage recorded. The transepithelial potential deviated no more than 1% from the voltage of the command step, while the current after the initial capacitive transient was not time dependent. All data were fitted to the Goldman-Hodgkin-Katz equation [2,3] using a non-linear least-squares curve fitting algorithm [9].

Results

Nystatin was added to the mucosal K_2SO_4/saline to remove functionally the apical membrane [6]. The serosal surface was bathed in the normal NaCl/saline. After the transepithelial potential and conductance had reached steady values, indicating the completion of nystatin action, voltage stimuli of various magnitudes were applied across the tissue, and the current responses recorded. Because of the nystatin action, these currents effectively represented the response of only the basolateral membrane. The current 100 ms after the beginning of the voltage step was measured and used to generate a current-voltage relationship. (Currents taken anytime after the capacitive transient produced identical *I-V* relationships.) This *I-V* relationship was then corrected for series and junctional resistance (see Methods) and the corrected *I-V* curve fitted to the constant-field equation [2,3]

$$I = \frac{F^2}{RT} P_K V \left\{ \frac{[K]_s + \frac{P_{Na}}{P_K}[Na]_s + \frac{P_{Cl}}{P_K}[Cl]_m - [K]_m + \frac{P_{Cl}}{P_K}[Cl]_s \, e^{-VF/RT}}{1 - e^{-VF/RT}} \right\} \quad (1)$$

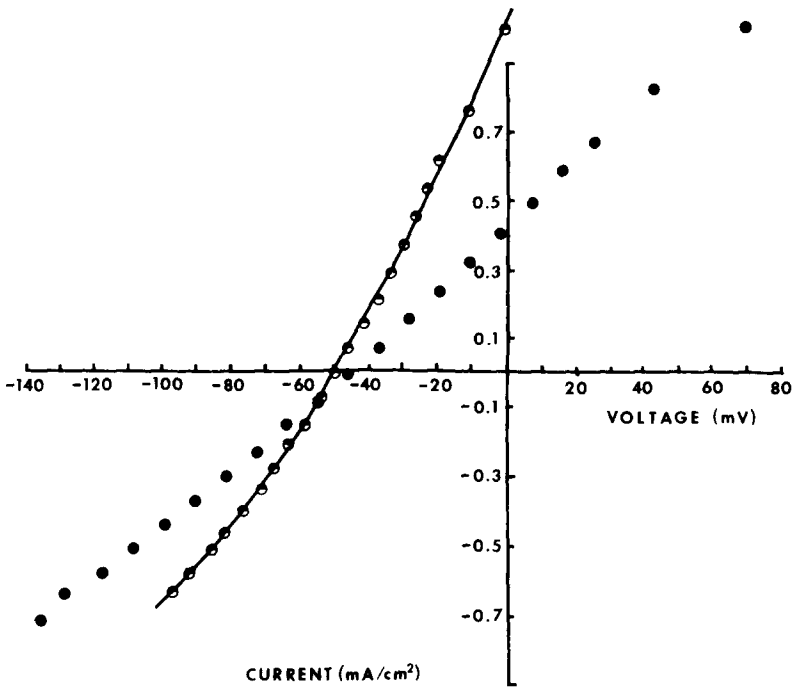

Fig. 1. Representative *I-V* relationship of the basolateral membrane of the rabbit descending colon. •, uncorrected data. ○, the *I-V* relationship after correction for series and junctional resistances. Line through the points is the best-fit curve to Eqn. 1.

where I is the current response to a step-potential change, V (reference to the serosal solution). Subscripts 'm' and 's' refer to the mucosal and serosal chamber, respectively. R, T and F have their usual meaning. Concentrations are in units of mM/cm^3 and P_K (potassium permeability) in cm/s. We should note that, strictly speaking, the voltage equation described by Goldman [2] and Hodgkin and Katz [3] does not depend upon the constant-field assumptions. However, the current equation described here does [10].

Fig. 1 illustrates the original and corrected data and the best-fit curve to the corrected data using the above equation. Three parameters were simultaneously fit: P_K, P_{Na}/P_K, and P_{Cl}/P_K.

Although, any reasonable preliminary estimate for the three parameters could have been used as a basis for the fitting procedure, to speed the convergence process, we chose as initial estimates the parameters given in Table I which were obtained from dilution potential and microelectrode experiments which will be reported elsewhere [7]. The final optimally fit values for three preparations are reported in Table I. Other values both larger and smaller than the final values were specifically chosen as poor initial estimates of the parameters. In all cases, although the convergence times were significantly longer, the final values were the same to the accuracy reported in the table.

To further investigate the *I-V* relationship of the basolateral membrane, Cs^+ was applied in the serosal solution. Cs^+ is noted in other tissues for its ability to

TABLE I

VALUES FOR PERMEABILITY RATIOS FROM CURRENT-VOLTAGE RELATIONSHIPS

Initial estimates used in the fitting procedure were $P_K = 1.20 \cdot 10^{-4}$ cm $\cdot$ s^{-1}, $P_{Na}/P_K = 0.04$, and $P_{Cl}/P_K = 0.06$.

Preparation No.	P_K (cm $\cdot$ s^{-1})	P_{Na}/P_K	P_{Cl}/P_K
1	$1.16 \cdot 10^{-4}$	0.048	0.061
2	$6.5 \cdot 10^{-5}$	0.062	0.05
3	$1.4 \cdot 10^{-5}$	0.09	0.013

block K^+ movement through the K^+-selective pathways [11,12]. It was of some comparative interest to determine whether Cs^+ could also block K^+ movement in the basolateral membrane of rabbit descending colon. Fig. 2 demonstrates that 7 mM CsCl added to the serosal NaCl/saline decreases the serosal to mucosal current flow. This reduction probably represents a reduction in K^+ current.

To determine if the reduction in serosal to mucosal current was a potential-dependent or current-dependent phenomenon, we altered the amount of current at any given potential by changing the zero-current potential. This alteration of the zero-current potential can be accomplished by changing the serosal K^+ concentration. If the Cs^+ blocking occurs at the same potential regardless of the amount of current, the blockage must depend only upon the membrane potential field which makes Cs^+ entry into the K^+ channel more favorable, rather than the K^+ current forcing Cs^+ into the pathway.

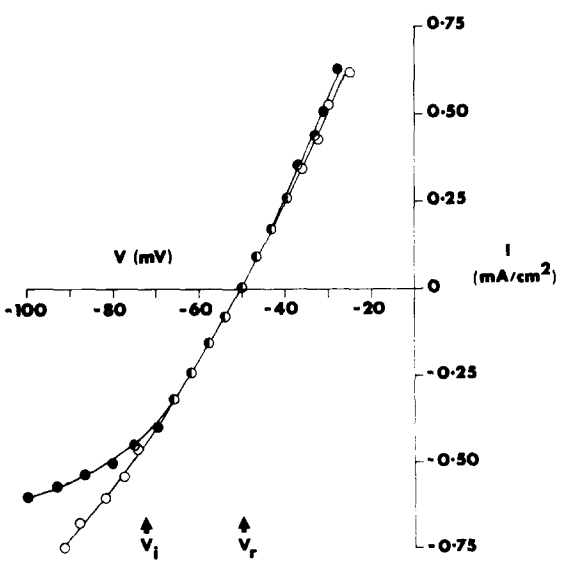

Fig. 2. Control (•) *I-V* curve is used to illustrate the increased rectification when 7 mM Cs^+ is added to the serosal solution (○). V_r, the resting membrane potential (serosal solution ground); V_i, the voltage at which the two *I-V* curves diverge. The addition of Cs^+ did not alter the resting membrane potential.

When the transepithelial zero-current potential is altered, the potential at which there is a noticeable deviation in the *I-V* relationship of the Cs^+-treated membrane from that of the untreated membrane occurs at 24 ± 2.2 (n = 7) mV more negative than the zero-current potential, but is independent of the value of the zero-current potential. This suggests that the Cs^+ blockage, while not dependent on current, is also not strictly dependent on potential, but rather, seems to depend upon the displacement of potential from the zero-current potential.

Discussion

From the results presented in Table I and Fig. 1, we conclude that the basolateral membrane of the rabbit descending colon may be adequately modeled on the basis of the Goldman-Hodgkin-Katz equation (Eqn. 1).

The question is whether the parameter values necessary to fit the data to this equation are reasonable. In this regard, the best evidence is the comparison of the values determined from the *I-V* relationship and those obtained by independent methods [7], which are given as the initial estimates in Table I. In the case of each parameter, the agreement is good, suggesting that values determined from the *I-V* relationship and Eqn. 1 are reasonable and that the basolateral conductance is adequately described by this equation.

If the permeabilities are correct, can they give us any information about the functioning of the rabbit colon in vivo? One point that seems clear is that the Cl^- permeability of the basolateral membrane seems quite low.

The ability of Cs^+ to block inward current in colon is similar to the blockage reported for other preparations as diverse as frog muscle [13], starfish egg [12] and *Aplysia* giant neuron [14].

From a comparative standpoint this similarity is interesting since it suggests, in some respects at least, that the basolateral membrane of epithelia is similar to the membranes of a variety of other cells. It would be consoling to think that the general permeability properties of many cell membranes are quite similar with the differences being due only to a few specific conductance or transport mechanisms.

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