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SHORT CIRCUIT CURRENT IN TIGHT AND LEAKY EPITHELIA

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Summary

The effect of short circuit current on the unidirectional fluxes of ions transported across tight and leaky epithelia was investigated. It was found that short circuiting of the frog gastric mucosa (classified as a tight epithelium) caused a decrease of the passive J_{ms}^{Cl} and a significant increase of the net Cl^- secretion. However, no significant change of H^+ secretory rate was observed. On the other hand, short circuiting of the mouse intestine (a known leaky membrane) caused a simultaneous increase of both J_{ms} and J_{sm} fluxes of Na^+ while the net fluxes of Na^+ and Cl^- remained unchanged. Also, short circuiting did not change the water permeability of the mouse intestine. To explain some of these results a theoretical model is presented to demonstrate that while short circuiting can block the passive ionic movement, it will cause an increase in the energy consumption of the system and introduce certain important changes in the ionic barriers and e.m.fs. The simultaneous increase in the unidirectional fluxes of Na^+ under short circuit conditions can best be explained by a decrease in the polarized nature of the transepithelial shunt, thereby increasing the diffusion coefficient of the ion(s). Such an increase is specially favorable to the Na^+ rather than an anion.

Introduction

In the study of ion transport across biological membranes Ussing and Zerahn [1] introduced the use of the short circuit current technique for the distinction between active and passive ion movement. Since then this technique has been used in the study of ion transport across a number of other biological membranes as well as the frog skin where it was first applied. On the basis of an equivalent circuit model it is determined that short circuiting will block the net passive ionic movement across the membrane and thereby the short circuit

current would be a measure of the net active ion movement. An implicit assumption is being made in developing this technique and that is, the membrane behaves like its electrical analog. Several investigators have questioned the applicability and validity of the use of the short circuit current as a measure of the active transport. Ginzburg and Hogg [2] showed that the short circuit current is equal to the pumping current only under special conditions. Furthermore, in a recent study, Rehm [3] demonstrated that the criteria for passive transport, as recognized by the Ussing school, are valid only if all the ions are transported via conductive pathways which can be represented in an equivalent circuit model, otherwise other information is needed. However, on the basis of irreversible thermodynamics Kedem [4] has shown that the measurement of the short circuit current, membrane potential and the rate of oxygen consumption are unequivocally important in the study of active ion transport. It was then obvious that the interest of some investigators pointed toward the use of the thermodynamic approach in studying ion transport as presented by Caplan and Essig [5] and Labarca et al. [6].

Our laboratory has used the short circuit technique in studying the 'ions' transport across the frog gastric mucosa [7] and mouse intestine [8,9]. Each of these membranes has relatively low resistance as compared with that of the frog skin or toad urinary bladder. Furthermore, they both possess a non-conductive ionic pathway for chloride and bicarbonate [8,10]. In view of the reservations and criticism of the short circuit technique, some of which were presented above, a question is raised as to whether the frog stomach and mouse intestine will respond differently under open circuit condition. Furthermore, since short circuiting is established when the algebraic sum of the mucosal and serosal potentials becomes zero, one may question the new status of the membrane diffusion barrier. Would this bring a change in the membrane activation energy? Such a change was indicated in our previous work on intracellular measurements [11]. It is also not clear whether this change in activation energy is due to an increase in oxygen consumption induced by short circuiting. The present work is designed to answer some of these questions with the aid of data obtained under open and short circuit conditions plus the solution of a two membrane electrical model with a variable shunt.

Methods

Two types of epithelial membranes were used in this study, frog stomach and mouse intestine. Two sets of measurements were obtained in each membrane experiment, one under short circuit condition, the other in open circuit condition. In the experiment with frog stomach, the stripped gastric mucosa from *Rana pipiens* was divided into two portions which were mounted in two identical Ussing chambers and incubated at room temperature (23°C). The mucosal side was bathed with a Cl^- secretory solution and the serosal side with a HCO_3^--buffered NaCl-Ringer solution [7]. The H^+ secretory rate was determined with a radiometer titrator and the Cl^- flux measurement was performed by adding ^{36}Cl in either side of the chamber. Samples of bathing solution from each side of the chamber were collected at 30-min intervals and counted in an automatic gas-flow beta Geiger counter. In the experiments with

mouse intestine, two adjacent sections of the upper jejunum of albino Swiss-Webster mouse were used. The sections were mounted in two identical Ussing chambers and bathed with mammalian Krebs-Ringer solution containing 5.5 mM D-glucose. The chambers were kept in incubators at a constant temperature of 37°C and gassed with 95—5% of O_2-CO_2 gas. For ionic flux measurements, ^{22}Na and ^{36}Cl were added into the bathing solution on one side of the membrane. Duplicate samples were collected at 30-min intervals. They were counted in an automatic Well scintillation counter and gas-flow beta Geiger counter. The details of the procedure have appeared in our previous publications [7,8,12].

In separate sets of experiments, tritiated water, ^3HHO, was used to determine the water permeability of mouse intestine under either open circuit or short circuit condition. The procedure has been described elsewhere [13].

Results

Chloride flux and H^+ secretory rate in frog stomach. Cl^- flux and H^+ secretory rate of the frog gastric mucosa were determined separately in 10 sets of experiments and the results are summarized in Table I. After a steady state was attained, the mucosa maintained an average potential of 25 mV with the serosal side being electropositive and an average transmembrane resistance of 200 ohm · cm^2. The average H^+ secretory rate was 2.92 μequiv. · h^{-1} · cm^{-2}. The average Cl^- flux values under open circuit condition were 3.18 μequiv. · h^{-1} · cm^{-2} for J_{ms} and 5.89 μequiv. · h^{-1} · cm^{-2} for J_{sm}, making a net Cl^- secretion of 2.71 μequiv. · h^{-1} · cm^{-2}. This value is very close to the H^+ secretory rate observed under the same condition. In another 8 pairs of gastric mucosa preparations under short circuit condition it was found there was a slight increase in J_{sm}^{Cl} and a very significant decrease in J_{ms}^{Cl} (average, 1.83 μequiv. · h^{-1} · cm^{-2}), thus causing a significant increase of the net Cl^- secretion, the J_{net}^{Cl} = 4.51 μequiv. · h^{-1} · cm^{-2}. By using this J_{net}^{Cl} value and the measured I_{sc}, the H^+ secretory rate was then calculated from Hogben's equation [14] and found to be 2.73 μequiv. · h^{-1} · cm^{-2}, which was not significantly different from the value observed under open circuit condition.

Na^+ and Cl^- fluxes in mouse intestine. When the mouse jejunum was bathed

TABLE I

^{36}Cl FLUX MEASUREMENT OF FROG GASTRIC MUCOSA

Condition	p^{2}H (mV)	J_{ms}^{Cl}	J_{sm}^{Cl}	J_{net}	I_{sc}	I_H
		(μequiv. · h^{-1} · cm^{-2})				
Open circuit	25	3.18 ± 0.28 (4) *	5.89 ± 0.91	2.72 ± 0.71	0	2.92 ± 0.24 (10) **
Short circuit	0	1.83 ± 0.12 (8)	6.22 ± 0.29	4.51 ± 0.25	1.78 ± 0.25	2.73 ***

* Mean ± S.E., the number in parenthesis represents the number of pairs of gastric mucosae, usually four 30-min periods of determination were conducted in each membrane.

** Values were obtained from direct titration in separated chambers.

*** Calculated from Hogben equation $I_H = I_{Cl} - I_{sc}$ (see ref. 14).

TABLE II

ION FLUX MEASUREMENTS IN MOUSE INTESTINE

Condition	p^2H (mV)	Ion	J_{sm}	J_{ms}	J_{net}	I_{sc}	J_R *
			(μequiv. $\cdot h^{-1} \cdot cm^{-2}$)				
Open circuit	2.7	Na^+	11.06 ± 0.7 (14) **	14.4 ± 0.76	3.34 ± 0.52	0	−8.00
		Cl^-	10.98 ± 0.54	6.32 ± 0.40	−4.66 ± 0.60	0	
Short circuit	0	Na^+	14.40 ± 0.38 (8)	17.62 ± 0.70	3.22 ± 0.30	1.33 ± 0.066	−6.46
		Cl^-	11.80 ± 0.40	7.23 ± 0.42	−4.57 ± 0.30		

* Residual flux, J_R, is calculated from : $I_{sc} - [J^{Na}_{net} - J^{Cl}_{net}]$.

** Mean ± S.E., the number in parenthesis represents the number of intestinal mucosa, usually four to five 30-min periods of determination were conducted in each membrane.

in Krebs-Ringer solution with 5.5 mM D-glucose, an average p^2H of 2.7 mV was observed with the serosal side being electropositive. The transmembrane resistance averaged 35 $\Omega \cdot cm^2$ after subtracting the solution resistance. Both unidirectional fluxes of Na^+, J^{Na}_{sm} and J^{Na}_{ms}, under short circuit condition were found to be much greater than the corresponding fluxes measured under open circuit condition. Results are summarized in Table II. It can be seen here that the J^{Na}_{ms} was 14.4 μequiv. $\cdot h^{-1} \cdot cm^{-2}$ under open circuiting, and increased to 17.6 under short circuiting; the J^{Na}_{sm} was 11.1 μequiv. $\cdot h^{-1} \cdot cm^{-2}$ under open circuiting and increased to 14.4 under short circuiting. However, the unidirectional Cl^- fluxes did not seem to be influenced very much by the short circuit condition. Furthermore, the net flux for either Na^+ or Cl^- was the same under open and short circuit condition. The calculated residual flux, J^R, was 8 μequiv. $\cdot h^{-1} \cdot cm^{-2}$ in open circuiting and 6.54 in short circuiting, a drop of 1.46 μequiv. $\cdot h^{-1} \cdot cm^{-2}$, which is very close to that of 1.33 μequiv. $\cdot h^{-1} \cdot cm^{-2}$ for the measured short circuit value, I_{sc}.

Water permeability in mouse intestine. In a total of 7 pairs of experiments with mouse intestine, the K_{trans} value for ^3HHO was measured. It was found that there was no significant difference of the water permeability constant under the open or short circuit condition. For example, the K_{trans} values for J_{ms} were 579.5 ± 21.9 $\cdot 10^{-7}$ cm $\cdot s^{-1}$ and 581.8 ± 29.0 $\cdot 10^{-7}$ cm $\cdot s^{-1}$, respectively, and the values for J_{sm} were 453.7 ± 24.7 $\cdot 10^{-7}$ and 513.9 ± 28.1 $\cdot 10^{-7}$ cm $\cdot s^{-1}$, respectively.

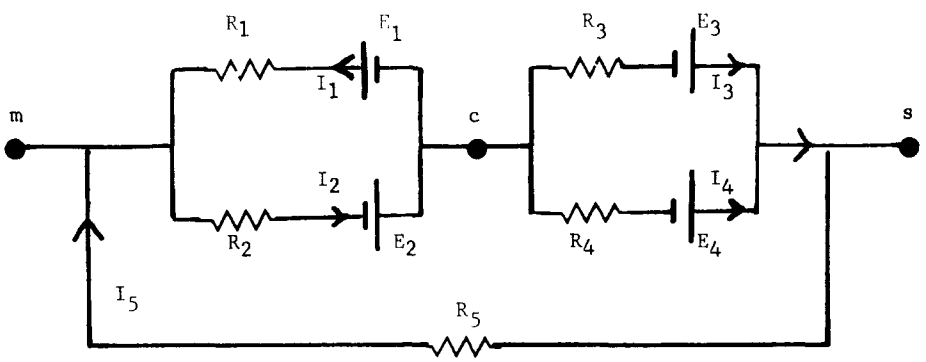
Discussion

The data obtained from our present study clearly demonstrates that short circuiting affects the unidirectional and net fluxes of ion(s) transported across the frog stomach and mouse intestine, in a rather complicated manner. The use of a purely passive electrical analog as postulated for the frog skin in the development of the short circuit technique is not sufficient to explain such findings. Therefore, we have chosen a different electrical analog, as shown in the diagram for Table III, which is a modified version of Rehm's model [3], as a working model for ion transport in gastric mucosa. As shown here, a set of values for each electrical parameter E or R is indicated, many of which were

obtained from our previous publication [7]. Based on these values we calculated the potentials across the membrane and the currents in the different pathways as a function of the internal shunt resistance, R_5, which is presumably responsible for most of the passive ion transport. When an epithelium membrane is tighter, the R_5 value is higher; when the membrane is more leaky, like the mouse intestine, the R_5 is lower. As the R_5 value approaches zero, the transmural potential, V_{sm}, decreases to zero, so the tissue is short circuited internally. Since it is not feasible to perform such a reduction of R_5 in a biological membrane, experimentally the transmural potential can be brought to zero by using an external field (short circuiting). It is evident then that the use of the short circuit method and the equivalent circuit implicitly assumes that the membrane and its electrical analog behave in a similar manner as described. Table III also shows that when the tissue is short circuited, the currents in all limbs are changing, V_{mc} increases, and V_{sc} decreases until it is equal to V_{mc}. The current, I_2, which is corresponding to the net Cl^- secretory rate in the gastric mucosa, has almost doubled after short circuiting. This coincides exactly what we have found in the net Cl^- measurement as presented in Table I. However, the current, I_1, which corresponds to the H^+ secretion in the gastric mucosa, decreases by almost 30% after short circuiting, while we found no change in the calculated H^+ secretory rate as shown in Table I. The entry of Cl^- across the serosal membrane of the gastric mucosa is through a neutral Cl^--HCO_3^- exchange pathway, as shown in the right loop of the diagram (Table III). The currents, I_3 and I_4, which are flowing

TABLE III

ELECTRICAL ANALOG OF CONDUCTIVE PATHWAYS IN GASTRIC MUCOSA



E_1 = 30 mV; E_2 = 7 mV; E_3 = 30 mV; E_4 = 28 mV; R_1 = 300 Ω; R_2 = 100 Ω; R_3 = 180 Ω; R_4 = 420 Ω.

R_5 (Ω)	V_{sm}	V_{mc}	V_{sc}	I_5	I_1	I_2	I_3	I_4
	(mV)			(μA)				
10^6	27	2	29	0.03	92.5	92.5	3.35	3.32
10^3	23	4	27	23	87	110	19	4
10^2	9	9	18	90	70	160	66	24
10	1.3	11.9	13.2	129	60	189	93	36
0	0	12.4	12.4	135 ($\approx I_{sc}$)	59	194	98	37

through the diffusion pathways for K^+ and Cl^-, respectively, are very minimal. Since there was no change in H^+ secretory rate after short circuiting, as observed in our data, it can be concluded that there is no significant change in the HCO_3^- flux to the serosal side. The increase of the net Cl^- secretion under short circuit current condition, as predicted from the theoretical model and observed from our direct measurement, can be explained by two possible mechanisms. First, there may be a passive shunt for Cl^- movement, either intercellularly or intracellularly. Thus, in the presence of the spontaneous transmural potential, V_{sm} (namely in the open circuit state), Cl^- is passively moved from mucosal to serosal as J_{ms}^{Cl}. Under the short circuit condition of $V_{sm} = 0$, such passive Cl^- movement is reduced to a minimal, as can be seen in our data that the J_{ms}^{Cl} reduced from 3.18 μequiv. $\cdot h^{-1} \cdot cm^{-2}$ in open circuiting to 1.83 μequiv. $\cdot h^{-1} \cdot cm^{-2}$ in short circuiting. Such a postulation is supported by the works of Forte [15] and Hogben [16], in which they reported there is a passive component for Cl^- flux of a similar magnitude. However, since neither J_{sm}^{Cl} nor the I_H was changed significantly after short circuiting, it is reasonable to conclude that the short circuit current gives a measure of the passive ionic flux across the membrane and does not affect the component of the active transport. An increase of the net transport rate results. The second possible mechanism is that short circuit current increases the metabolic process of the biological membrane resulting in an increase of ionic flux. In their experiment with frog skin, Ussing and Zerahn [1] reported an increase in Na^+ flux after short circuiting, from a value of 80 mC $\cdot h^{-1} \cdot cm^{-2}$ to a value of 178 mC $\cdot h^{-1} \cdot cm^{-2}$. Zerahn [17] measured the Na^+ flux as related to the oxygen consumption of the frog skin under different electrochemical gradients and found that the ratio of Na^+/O stayed constant as the electrochemical gradients varied. His data indicate that an increase in Na^+ transport is accompanied with an increase in oxygen consumption. Recently, more precise experimental data on other biological membranes obtained by several investigators has further supported such a conclusion. For example, Lang et al. [18], Labarca et al. [6] and Nellanus and Finn [19] reported that the increased Na^+ transport is associated with an increase of oxygen consumption by the metabolic process. Their data suggest that the short circuit current changes the thermodynamic state of the system. This could be partially inferred from the data in Table III, where the net energy rate supplied by the e.m.f. value being assigned here has doubled from 3.43 μW in open circuiting to 7.13 μW in the short circuiting condition. Since there is no external source of energy supplied in this system, the additional energy had to be from the system. Therefore, with the use of a non-equilibrium thermodynamic phenomenon one can explain that the increase in net Cl^- secretion under short circuiting is due to an increase in the Cl^- pump potency. The latter increase is attributed to a change in both energy and permeability components of the Cl^- pump. Using the equation of Linderholm [20] one can calculate the e.m.f. for the Cl^- pump in the frog stomach. As shown in Table IV, the E_{Cl} is 38.8 mV under the open circuit condition and decreases to 31.1 mV under the short circuit condition. Hence, an increase in Cl^- secretion could be interpreted as a decrease in the Cl^- diffusion barrier.

In the experiments with mouse intestine, the data presented here are more complicated than those observed in frog stomach. Here, short circuiting caused

TABLE IV

ACTIVE TRANSPORT POTENTIALS *

Membrane	Conditions	p^2H	E_{Na}	E_{Cl}
		(mV)		
Mouse intestine	Open circuit	2.67	9.72	17.4
	Short circuit	0	5.67	12.6
Frog stomach	Open circuit	25.0	—	38.8 **
	Short circuit	0	—	31.1

* Calculated from Linderholm's equation: $E_i = (V_s - V_m) - RT/Z_iF \ln J_{sm}/J_{ms}$.
** Compared to 35 mV obtained with a different method (see ref. 7).

an increase of the unidirectional fluxes of both Na^+ and Cl^- and this is contradictory to the expectation as observed from the frog stomach data. But one has to realize that the transmembrane potential and resistance of the intestinal membrane are very low and the tissue almost short circuits itself internally. Therefore, it is expected that the net flux of both Na^+ and Cl^- is unchanged under short circuit condition. This observation is further supported by the measurement of water permeability of the intestine where no significant change in the K_{trans} values was found under short circuit condition.

In Table II, the residual flux value, J_R, is equivalent to the net HCO_3^- being absorbed from the intestine [8]. Under open circuiting the J_R is 8 μequiv. $\cdot$ $h^{-1} \cdot cm^{-2}$ and decreases by 1.46 after short circuiting which is almost equal to the amount of current being used to short circuit the membrane, 1.38 μequiv. $\cdot$ $h^{-1} \cdot cm^{-2}$. Therefore, the conclusion is warranted that there is an amount of at least 1.46 μequiv. HCO_3^- being passive absorbed across the intestinal membrane which can be blocked by the short circuiting process. So, the short circuit current gives a measure of the passive component of ion(s) flux similar to the mechanism for the decrease of J_{ms}^{Cl} in gastric mucosa.

Table IV presents the data on E_{Na} and E_{Cl} of mouse intestine as calculated with the Linderholm equation [20]. It is evident that both E_{Na} and E_{Cl} fall considerably after short circuiting. The transport buarrier for Na^+ and Cl^- can be expressed mathematically as the ratio of the calculated e.m.f. divided by the net flux of that ion. It can be seen here that short circuiting drops the Na^+ barrier by 43% and the Cl^- barrier by 25%, while the net flux of both Na^+ and Cl^- was unchanged, as shown in Table II. Since the mouse intestine is a very leaky membrane, one would expect that the transepithelial shunt plays a major role in any change of ionic fluxes. We postulate that short circuiting causes a decrease in the polarized nature of the transepithelial shunt and thus induces an increase in the diffusion coefficient of the ions. Such an increase is especially favorable to Na^+ rather than an anion such as Cl^-, because it is believed that these channels are lined with negative charges.

Our conclusion that short circuiting causes some changes in the properties of the transepithelial shunt is further supported by the works of Armstrong et al. [21] on the bullfrog intestine and of Garcia-Diaz and Corcia [22] on the rat jejunum. They demonstrated that osmotic change across the tissue induces changes in the electrical properties of the transepithelial shunt.

It should be pointed out here that we have ignored the possible role of the sodium-glucose co-transport system on ion transport at the brush border of the intestinal epithelium. Since an increase of Na^+ entry at the brush border membrane by short circuiting would inactivate such a co-transport system and initiate an increase of glucose transport and oxygen consumption, it is difficult to give a convincing assessment on the nature and mechanism of ion transport across the intestine by the use of the short circuit current technique.

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