CAPACITATIVE TRANSIENTS IN VOLTAGE-CLAMPED EPITHELIA

J. F. GARCIA-DIAZ AND A. ESSIG
Department of Physiology, Boston University School of Medicine, Boston, Massachusetts 02118

ABSTRACT In voltage-clamped epithelia the cell membrane potential transient during a +10-mV transepithelial pulse conforms to the expected behavior for a series combination of two linear resistance-capacitance (RC) circuits. The evolution of the cell potential is characterized by a single time constant with values of 30–130 ms in frog skin and *Necturus* gallbladder. These observations have important consequences for the measurement of cell membrane resistance ratios and the interpretation of current-voltage relations.

INTRODUCTION

The assessment of ionic transport mechanisms in epithelia requires a precise knowledge of the cell membrane resistances, among other parameters. This is usually obtained by combining measurements of the cell resistance ($R_a + R_b$) and the so-called fractional apical resistance, $f_a^R = R_a/(R_a + R_b)$, where R_a and R_b are the lumped equivalent resistances of the apical and basolateral membranes. In voltage-clamped epithelia, f_a^R is measured by applying a brief transepithelial voltage pulse, ΔV_T, from the holding voltage and recording the deflection induced in the apical membrane potential, ΔV_a. The ratio $\Delta V_a/\Delta V_T$ provides a good estimate of f_a^R when most of the extracellular pathway resistance is localized at the tight junction and the system can be treated as a lumped equivalent circuit (Boulpaep and Sackin, 1980; Essig, 1982). For the accurate determination of f_a^R, ΔV_a has to be measured after the dissipation of the transients associated with the membrane capacitances. On the other hand, early measurement of ΔV_a is required in order to minimize possible changes in membrane resistances, cell electromotive forces, and/or polarization phenomena induced by the transepithelial pulse. The same considerations influence the interpretation of the current-voltage (I-V) relations of the cell membranes. Thus, a knowledge of the nature of capacitative transients is of fundamental importance.

DeLong and Civan (1984) have recently pointed out a method for the analysis of capacitative transients from the time course of the cell membrane potential after a step change in transepithelial voltage. Here we show that, using a +10-mV ΔV_T pulse, the time courses of ΔV_a in frog skin and *Necturus* gallbladder conform to the expected behavior for a series combination of two linear resistance-capacitance (RC) circuits with a combined time constant in the range of 30–130 ms.

CIRCUIT ANALYSIS

For the circuit depicted in Fig. 1 *A*, the change in apical membrane potential, $\Delta V_a(t)$, after a step change in the transepithelial clamping voltage, ΔV_T, is given by (see DeLong and Civan, 1984)

$$\Delta V_a(t) = \Delta V_T \frac{R_a}{R_a + R_b} + \Delta V_T \left(\frac{C_b}{C_a + C_b} - \frac{R_a}{R_a + R_b} \right) e^{-t/\tau}, \quad (1)$$

where

$$\tau = (C_a + C_b) \frac{R_a R_b}{R_a + R_b}. \quad (2)$$

That is, $\Delta V_a(t)$ will exhibit a single time constant, τ. The inclusion of a purely resistive extracellular shunt pathway in this circuit does not affect the above equations.[1] The amplitude of the exponential term in Eq. 1 can be written

$$\Delta V_T \frac{\tau_b - \tau_a}{(C_a + C_b)(R_a + R_b)}, \quad (3)$$

where $\tau_a = R_a C_a$ and $\tau_b = R_b C_b$. The sign of the exponential term depends then on the relative values of τ_a and τ_b. As shown by DeLong and Civan (1984), this gives rise to three possible times courses for $\Delta V_a(t)$ (see Fig. 1 *B*). The initial and steady state values of $\Delta V_a(t)$ are determined by the capacitances and resistances of the circuit, respectively. The initial value ($t = 0$) provides the basolateral fractional capacitance, f_b^C

$$f_b^C \equiv \frac{\Delta V_a(0)}{\Delta V_T} = \frac{C_b}{C_a + C_b} \quad (4)$$

[1]Note that in current-clamp mode the cell potential follows a double exponential time course in the presence of a finite resistance shunt (Suzuki et al., 1982).

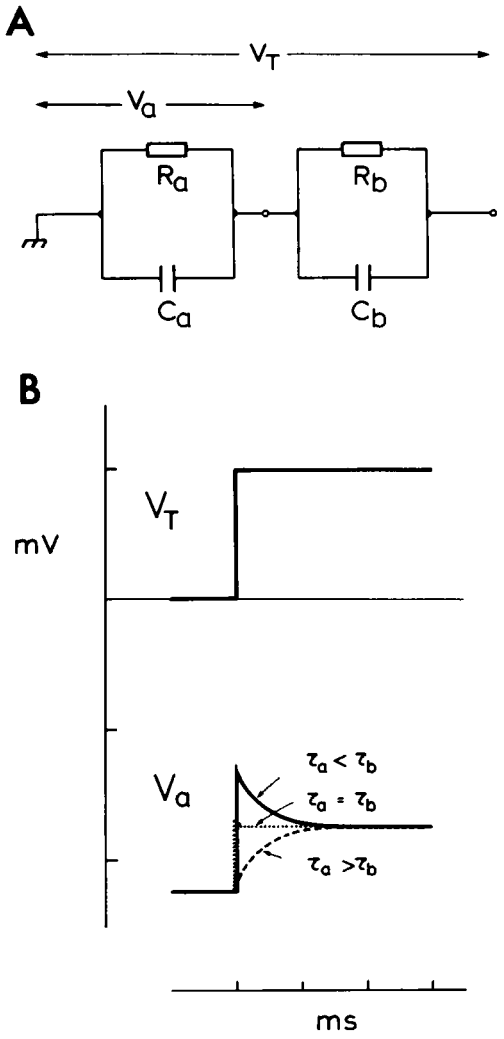

FIGURE 1 (*A*) Equivalent circuit of the epithelial cell. (*B*) Possible time courses of $\Delta V_a(t)$ after a step change in V_T. In this graph it is implicitly assumed that f_a^R is the same in all cases.

and the steady state ($t \rightarrow \infty$) gives the apical fractional resistance, f_a^R

$$f_a^R \equiv \frac{\Delta V_a(\infty)}{\Delta V_T} = \frac{R_a}{R_a + R_b}. \quad (5)$$

RESULTS AND DISCUSSION

The experimental details and description of the recording instrumentation are given in previous publications (Garcia-Diaz et al., 1983; Nagel et al., 1983). The settling time of our voltage clamp was ≃5 ms. The earliest $\Delta V_a(t)$ points collected for analysis were taken 10 ms after the onset of the command pulse.

Fig. 2 shows the typical response of $V_a(t)$ in frog skin to a +10-mV ΔV_T pulse from the short-circuit state ($V_T = 0$) (*a*) during control and (*b*) after mucosal addition of amiloride. According to the previous analysis, the time course exhibited by $\Delta V_a(t)$ in the control state indicates that $\tau_a < \tau_b$. Furthermore, as predicted by Eq. 1, $\Delta V_a(t)$ is

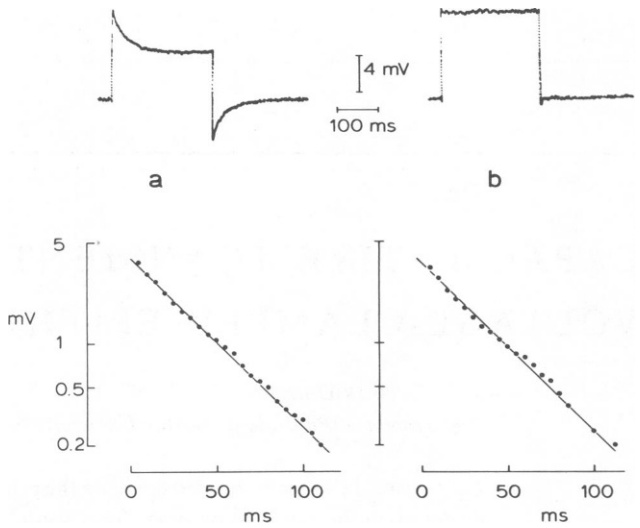

FIGURE 2 *Top:* Oscilloscope recordings of $\Delta V_a(t)$ in frog skin during a +10-mV ΔV_T pulse (250 ms) from the short-circuited state. (*a*) Control conditions; (*b*) after mucosal addition of 20 μM amiloride. *Bottom:* Semilogarithmic plots of the on (*left*) and off (*right*) decays for the $\Delta V_a(t)$ shown in *a*. Time constants were the same in both cases: 37 ms.

characterized by a single time constant, which is the same for the on and off relaxations (Fig. 2, bottom). The symmetry of the on and off responses supports the interpretation that they are due to charging of linear R-C elements, and not to time-variant resistances (Mauro, 1961). Although the cell membrane resistance ratio of frog skin is voltage dependent, the half-times for this effect are in the order of seconds (Nagel and Essig, 1982). In contrast, in the five frogs studied τ ranged from 38 to 87 ms (Table I, second column). In agreement with our values, Tang and Helman (1984) reported a mean value of 42 ms for the fast exponential associated with capacitative transients in frog skin. By extrapolation of the semilogarithmic plots to $t = 0$, it is possible to estimate the fractional capacitance, f_b^C. This was always >0.9 (Table I, third column). This observation explains why in the presence of amiloride, $\Delta V_a(t)$ shows a square-wave response (Fig. 2 *b*), since after addition of the drug R_a becomes $\gg R_b$, so that $R_a/(R_a + R_b)$ approximates 1. Thus the amplitude of the exponential term in Eq. 1 approaches zero and $\Delta V_a(t)$ exhibits a square wave response irrespective of the value of τ.

Combining f_a^R with the cell conductance, $g_c = 1/(R_a + R_b)$, measured as the amiloride inhibitable transepithelial conductance,[2] one can calculate R_b:

$$R_b = (1 - f_a^R)/g_c \quad (6)$$

[2]This method for measuring g_c relies on the assumption that the shunt pathway resistance is unaffected by amiloride, which is not always the case (Nagel et al., 1983). To reduce possible effects of amiloride on extracellular resistance we employed a $NaNO_3$ Ringer's solution as the apical solution (Nagel et al., 1983).

TABLE 1
TIME CONSTANT AND CELL CAPACITANCES IN FROG SKIN

Skin	τ	f_b^C	f_a^R	R_b	C_a	C_b	V_a	V_a'	$f_a^{R'}$
	ms			$k\Omega \cdot cm^2$	$\mu F/cm^2$	$\mu F/cm^2$		*mV*	
1	38	0.97	0.53	2.35	0.9	29	−40	− 94	0.96
2	72	0.93	0.53	3.36	2.8	37	−53	−106	1.00
3	87	0.98	0.52	4.80	0.7	34	−45	−102	0.98
4	72	0.91	0.37	4.20	4.1	42	−32	−104	0.99
5	58	0.93	0.41	4.90	2.0	27	−23	−100	0.99
Mean		0.94			2.1	34			0.98
SD		0.03			1.4	6			0.02

For comparison purposes we include values of V_a before and ~1–2 min after (V_a') addition of 20 μM amiloride to the apical solution, as well as f_a^R in the presence of amiloride ($f_a^{R'}$).

and, from Eq. 2, the sum $C_a + C_b$

$$C_a + C_b = \tau/(R_b \cdot f_a^R) . \quad (7)$$

The individual capacitances can now be estimated using the measured value of f_b^C. These are shown in Table I. For comparison, previous estimates of C_a and C_b in frog skin derived from impedance analysis, were 1.6 and 78 $\mu F/cm^2$ (Smith, 1971) and 2 and 16 $\mu F/cm^2$ (Cuthbert and Painter, 1969). The large value of C_b per tissue area in this epithelium is probably a consequence of both the basolateral membrane folding and the multilayered syncytial structure.

Further support for the presence of epithelial capacitative transients with τ of the order of tens of milliseconds stems from experiments in *Necturus* gallbladder. In this single-layered epithelium, where the mucosal membrane exhibits multiple long projections (Suzuki and Frömter, 1977), the time course of $\Delta V_a(t)$ at short circuit can follow any of the patterns depicted in Fig. 1 *B*. Interestingly, the response can be altered by changing the holding V_T. Fig. 3 shows $V_a(t)$ after a +10-mV ΔV_T pulse from (*a*) $V_T = 0$ and (*b*) ~1 min after V_T was clamped to +50 mV. The time course of $\Delta V_a(t)$ in *a* indicates that at short circuit $\tau_a \gtrsim \tau_b$ for this particular preparation. After clamping V_T to a holding voltage of +50 mV, R_a decreases as a consequence of the voltage-dependent apical K conductance (Garcia-Diaz et al., 1983). Now τ_a becomes $<\tau_b$ and the time course of $\Delta V_a(t)$ changes accordingly. As was the case for the frog skin, the time course of $\Delta V_a(t)$ follows a single exponential, the same for the on and off responses.

The voltage sensitivity of R_a in *Necturus* gallbladder can be used to demonstrate the dependence of τ on f_a^R predicted by Eq. 7. By clamping V_T to increasingly positive values R_a is decreased; if R_b and the *C*'s remain constant, τ should be proportional to f_a^R. The results of such an experiment are shown in Fig. 4. *A* shows the dependence of f_a^R on the holding V_T. The insets are the oscilloscope traces of $\Delta V_a(t)$ after a +10-mV ΔV_T pulse from the holding V_T. The amplitudes of the exponential decays were large enough from V_T = +30 to +70 mV to allow calculation of τ. *B* shows the relation between τ and f_a^R for these five holding voltages. The near proportionality of this relation supports our previous claim that the voltage sensitivity of f_a^R in gallbladder epithelium is due to the apical membrane (Garcia-Diaz et al., 1983). Also shown in Fig. 4 (*C*) are the values of f_b^C determined at each holding V_T by extrapolation of the semilogarithmic plots of $\Delta V_a(t)$ to $t = 0$. f_b^C remains near constant at an approximate value of 0.7. As expected from Eq. 1, $\Delta V_a(t)$ exhibits a square wave time course when $f_a^R = f_b^C$, which for this particular experiment happened at a holding V_T between 0 and +20 mV. Note that the voltage-induced change in R_a has a half-time of several seconds (Garcia-Diaz et al., 1983) and thus will not

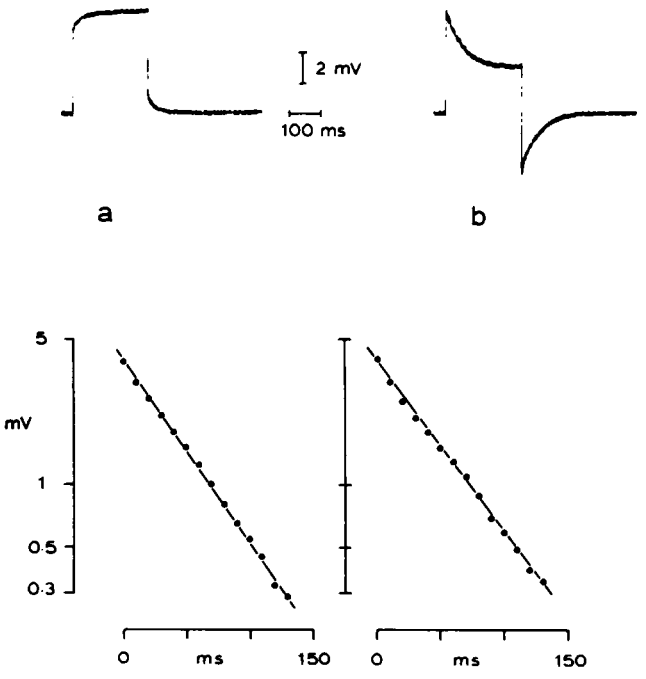

FIGURE 3 *Top:* Oscilloscope recordings of $\Delta V_a(t)$ in *Necturus* gallbladder during a +10-mV ΔV_T pulse (250 ms) from a holding $V_T = 0$ (*a*) and V_T = 50 mV (*b*). *Bottom:* Semilogarithmic plots of the on (*left*) and off (*right*) decays for the $\Delta V_a(t)$ shown in *b*. Time constants were 51.5 ms (on) and 52.9 ms (off).

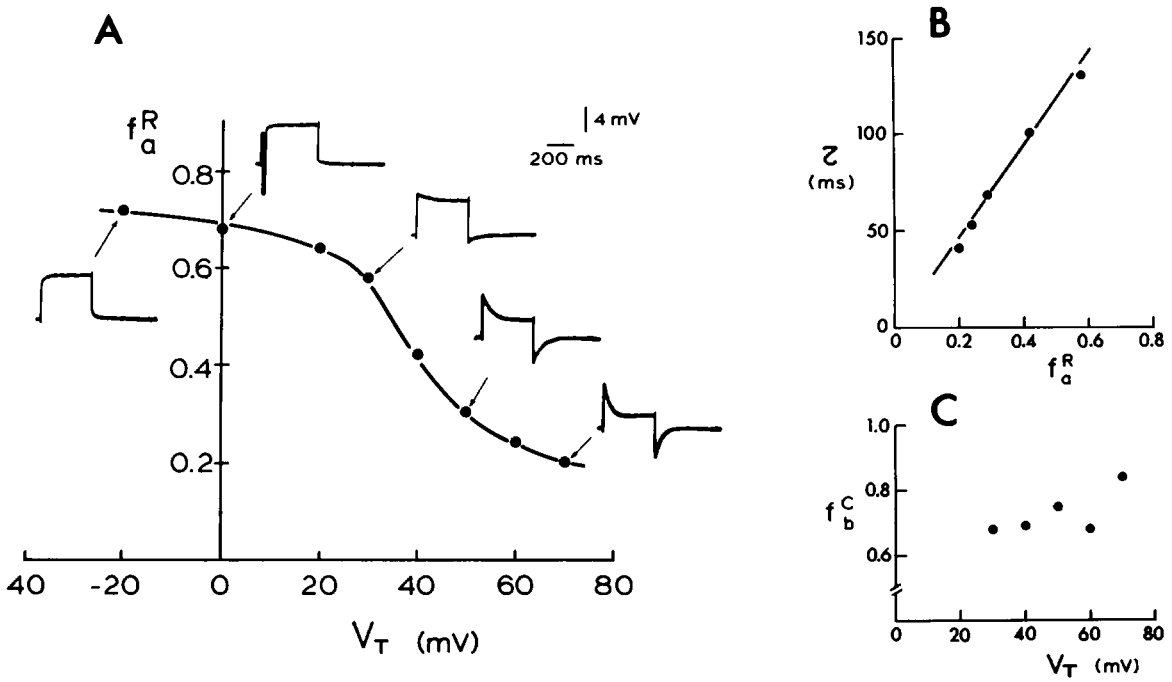

FIGURE 4 (*A*) Dependence of apical fractional resistance, f_a^R, on transepithelial holding voltage, V_T, in *Necturus* gallbladder. The insets show the oscilloscope traces of $\Delta V_a(t)$ for several holding V_T. (*B*) Relation between τ and f_a^R for V_T between 30 and 70 mV from the experiment shown in *A*. (*C*) f_b^C as a function of V_T (between 30 and 70 mV). After crossing the epithelium f_a^R was 0.96 in this experiment. The data in this figure were not corrected for this factor.

interfere significantly with the capacitative transients discussed here.

All of the above observations strongly suggest that we are dealing with capacitative transients with time constants in the range 30–130 ms, and not with other types of time-dependent phenomena.[3] Voltage gating appears unlikely, since although first-order transitions imply single-exponential relaxation after a step voltage perturbation, all the well-studied voltage-sensitive channels are more complex (Hille, 1984, p. 330). It might be argued that the nature of the gating response is dependent on the holding voltage V_T, such that a positive perturbation ΔV_T opens channels above the inversion point ($V_T \simeq$ 0–20 mV), but closes channels at lower settings of V_T. But if this were the case we would not observe a monotonic dependence of f_a^R on V_T as in Fig. 4 *A*. Concentration polarization and alteration of cell emf's can indeed occur, but their time constants would be expected to be much longer and their magnitudes dependent on V_T (Tang and Helman, 1984), so that with the brief 10-mV perturbations employed here these effects should be unimportant.

Our observations have important consequences not only for the proper measurement of f_a^R, but also for the interpretation of the I-V relations of the cell membranes. As we indicated previously (Garcia-Diaz and Essig, 1985), for epithelia where $\tau_a \ll \tau$, the relation between apical voltage and cell current, I_c, at any time after the onset of the V_T pulse is determined solely by R_a, i.e.,

$$\frac{\Delta V_a(t)}{\Delta I_c(t)} = -R_a . \tag{8}$$

Thus, under these conditions the apical I-V relation can be analyzed soon after the onset of the V_T pulse, before the development of polarization effects, in spite of the presence of capacitative transients. However, unless measurements are made after the dissipation of capacitative transients, the basolateral I-V relationship will underestimate R_b, and $\Delta V_a/\Delta V_T$ will overestimate f_a^R (DeLong and Civan, 1984; Schultz et al., 1984).

Finally we would like to point out that the method outlined here may well be useful in the measurement of the capacitances and resistances of epithelial cell membranes, if as in the case of frog skin, an independent measurement of cell resistance is possible. This approach is simpler than impedance analysis.

[3]DeLong and Civan (1984) and Schultz et al. (1984), working with the split frog skin and *Necturus* urinary bladder, respectively, have presented the view that current-voltage relationships during this interval are not distorted significantly by capacitative effects. Their observations, however, do not support this claim, as we have indicated elsewhere (Garcia-Diaz and Essig, 1985).

This work was supported by National Institute of Health Grant AM 29968.

Received for publication 18 February 1985 and in final form 18 April 1985.

REFERENCES

Boulpaep, E. L., and H. Sackin. 1980. Electrical analysis of intraepithelial barriers. *Curr. Top. Membr. Transp.* 13:169–197.

Cuthbert, A. W., and E. Painter. 1969. The action of antidiuretic hormone on cell membranes. Voltage transient studies. *Br. J. Pharmacol.* 35:29–50.

DeLong, J., and M. M. Civan. 1984. Apical sodium entry in split frog skin: current-voltage relationship. *J. Membr. Biol.* 82:25–40.

Essig, A. 1982. Influence of cellular and paracellular conductance patterns on epithelial transport and metabolism. *Biophys. J.* 38:143–152.

Garcia-Diaz, J. F., and A. Essig. 1985. Time-dependent phenomena in voltage-clamped epithelia. J. Membrane Biol. In Press.

Garcia-Diaz, J. F., W. Nagel, and A. Essig. 1983. Voltage-dependent K conductance at the apical membrane of *Necturus* gallbladder. *Biophys. J.* 43:269–278.

Hille, B. 1984. Ionic Channels of Excitable Membranes. Sinauer Assoc., Sunderland, MA. 426 pp.

Mauro, A. 1961. Anomalous impedance, a phenomenological property of time-variant resistance. An analytic review. *Biophys. J.* 1:353–372.

Nagel, W., and A. Essig. 1982. Relationship of transepithelial electrical potential to membrane potentials and conductance ratios in frog skin. *J. Membr. Biol.* 69:125–136.

Nagel, W., J. F. Garcia-Diaz, and A. Essig. 1983. Contribution of junctional conductance to the cellular voltage-divider ratio in frog skins. *Pfluegers Arch. Eur. J. Physiol.* 399:336–341.

Schultz, S. G., S. M. Thompson, R. Hudson, R., S. R. Thomas, and Y. Suzuki. 1984. Electrophysiology of *Necturus* urinary bladder. II. Time-dependent current-voltage relations of the basolateral membranes. *J. Membr. Biol.* 79:257–269.

Smith, P. G. 1971. The low frequency electrical impedance of the isolated frog skin. *Acta Physiol. Scand.* 81:355–366.

Suzuki, K., and E. Frömter. 1977. The potential and resistance profile of *Necturus* gallbladder cells. *Pfluegers Arch. Eur. J. Physiol.* 371:109–117.

Suzuki, K., G. Kottra, L. Kampmann, and E. Frömter. 1982. Square wave pulse analysis of cellular and paracellular conductance pathways in *Necturus* gallbladder epithelium. *Pfluegers Arch. Eur. J. Physiol.* 394:302–312.

Tang, J., and S. I. Helman. 1984. Kinetics of change of intracellular voltage in response to voltage clamping epithelia of frog skin. *Fed. Proc.* 43:445. (Abstr.)