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Voltage- and Time Dependence of Apical Membrane Conductance During Current Clamp in *Necturus* Gallbladder Epithelium

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Summary. The effects of short (1 sec) and long (1 min) transepithelial current clamps on membrane voltages and resistances of *Necturus* gallbladder were investigated. Transepithelial and cell membrane current-voltage relationships determined from 1-sec clamps revealed that: a) depolarization of the apical membrane voltage (V_{mc}) results in a marked decrease in apical membrane fractional resistance (fR_a), whereas hyperpolarization of V_{mc} results in either no change in fR_a or a small increase, and b) the voltage-dependent changes in fR_a are essentially complete within 500 msec. Exposure of the tissue to 5 mM TEA^+ on the mucosal side caused no significant change in baseline V_{mc} (-69 ± 2 mV) and yet virtually abolished the voltage dependence of fR_a. A possible interpretation of these results is that two types of K^+ channels exist in the apical membrane, with different voltage dependencies and TEA^+ sensitivities. Acidification or Ba^{2+} addition to the mucosal solution also reduced the voltage-dependent changes in fR_a. The time courses of the changes in fR_a and in the cable properties of the epithelium were assessed during 1-min transepithelial current clamps ($\pm$ 200 $\mu A/cm^2$). No secondary change in fR_a was observed with mucosa-to-serosa currents, but a slow TEA^+-sensitive decrease in fR_a (half-time of seconds) was evident with serosa-to-mucosa currents. Cable analysis experiments demonstrated that the initial (<500 msec) voltage-dependent decrease in fR_a is due to a fall in apical membrane resistance. The later decrease in fR_a is due to changes in both cell membrane resistances attributable to the increase in transcellular current flow resulting from a fall in paracellular conductance. The voltage dependence of the apical membrane conductance is a more significant problem in estimating fR_a than the current-induced effects on the lateral intercellular spaces. In principle, TEA^+ can be used to prevent the nonlinear behavior of R_a during measurements of the voltage divider or membrane resistance ratio.

Key Words voltage-dependent K^+ channel · TEA^+ · barium · circuit analysis · cable analysis · current-voltage relationship

Introduction

Measurement of the individual apical and basolateral cell membrane resistances is essential in electrophysiological studies designed to evaluate the mechanisms responsible for salt and fluid transport in epithelia. In *Necturus* gallbladder epithelium, estimates of the cell membrane resistances have been obtained utilizing AC-impedance analysis (Kottra & Frömter, 1984*a*,*b*), flat-sheet intracellular cable analysis (Frömter, 1972; Reuss & Finn, 1975*a*), or variations of these techniques including square-wave pulse analysis (Suzuki et al., 1982) and two-point intracellular cable analysis (Bello-Reuss, Grady & Reuss, 1981; Petersen & Reuss, 1985; and *see* Materials and Methods). Regardless of the approach used, voltage-dependent and/or time-dependent alterations in cell membrane resistances during the measurement or during experimental perturbations can mask expected changes in resistance and give misleading results. For example, we have recently demonstrated that elevation of serosal solution [K^+] causes an expected depolarization of basolateral membrane voltage, but also an unexpected decrease in the apical membrane fractional resistance (fR_a) (Stoddard & Reuss, 1988). This "anomalous" fall in fR_a can be explained in terms of a voltage-dependent decrease in apical membrane resistance (R_a), first described in *Necturus* gallbladder by García-Díaz, Nagel and Essig (1983), and also found in guinea-pig gallbladder (Gunter-Smith, 1987).

The present studies were designed to investigate the voltage- and time dependence of the cell membrane resistances under current-clamp conditions by simultaneously measuring fR_a and performing two-point intracellular cable analysis. The combination of these measurements allows for determination of the relative changes in apical and basolateral membrane resistances. In agreement with García-Díaz et al. (1983), our results further support the notion that the voltage-dependent decrease in fR_a is largely attributable to a fall in R_a. However, we find that the decrease in fR_a consists of two distinct phases. The first occurs with an estimated half-time of $\leq$ 100 msec and is due to a voltage-dependent decrease in R_a. The second has a

half-time on the order of seconds and can be attributed to: 1) a further fall in R_a attributable to a secondary depolarization of V_{mc}, and 2) an increase in basolateral membrane resistance. Both slow changes stem from an increase in transcellular current flow due to a clamp-induced elevation in paracellular resistance.

Materials and Methods

Mudpuppies (*Necturus maculosus*) were obtained from Nasco Biologicals (Ft. Atkinson, WI) or Kon's Scientific (Germantown, WI) and kept in a large refrigerated aquarium (5–10°C). After anesthetizing the animal with tricaine methanesulfonate and removing the gallbladder, the tissue was mounted horizontally in a modified Ussing chamber as described previously (Reuss & Finn, 1975*a*; Weinman & Reuss, 1984). The epithelium was bathed symmetrically with the control bathing solution which was continuously exchanged and had the following ionic composition (in mM): 90 NaCl, 10 $NaHCO_3$, 2.5 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, and 0.5 NaH_2PO_4. This solution was equilibrated with a gas mixture of 1% CO_2/99% air and had a pH of ~7.65. The TEA^+ Ringer's solution was prepared by equimolar replacement of NaCl with tetraethylammonium chloride (5 mM). Ba^{2+} Ringer's was made by addition of 5 mM $BaCl_2$. Low pH Ringer's (pH = 6.65) was prepared by isomolar substitution of HCO_3^- with Cl^- (9 mM).

Electrical Measurements

Measurements of transepithelial voltage (V_{ms}) and cell membrane voltages (apical, V_{mc}; basolateral, V_{cs}) were made using techniques similar to those previously described (Reuss & Finn, 1975*a,b*; Stoddard & Reuss, 1988). Both V_{ms} and V_{cs} were referred to serosal ground which consisted of an Ag-AgCl pellet separated from the solution by a short Ringer's-agar bridge. Subtraction of V_{ms} from V_{cs} gave V_{mc}. The mucosal electrode was a calomel half-cell connected to the bathing solution by a flowing saturated-KCl bridge. This arrangement minimized liquid junction potentials arising from changes in the composition of the mucosal solution.

Intracellular microelectrodes were pulled from inner-fiber borosilicate glass (1 mm OD, 0.5 mm ID; Glass Company of America, Millville, NJ) on a horizontal puller (model PD-5; Narishige, Japan) and backfilled with 1 M KCl. Tip resistances ranged between 20 and 100 MΩ when measured in Ringer's. Final advancement of the microelectrode into the cell was done with a hydraulic micromanipulator (model MO-103, Narishige, Japan). Cells were impaled across their apical membranes at an ~60° angle, and impalements were validated using criteria previously described (Reuss & Finn, 1975*a*; Reuss & Weinman, 1979; Weinman & Reuss, 1984).

Current-Voltage Relationships

Transepithelial and cell membrane I_t-V relationships were determined from the voltage changes elicited by calibrated transepithelial constant-current pulses (I_t) of 1-sec duration, applied via Ag-AgCl electrodes at intervals of 6 to 10 sec. Starting at 50 $\mu A/cm^2$, I_t was passed alternately in the mucosa-to-serosa (*m*-to-*s*) and serosa-to-mucosa (*s*-to-*m*) directions over a range of ±450 $\mu A/cm^2$. The voltage changes were measured from averages of digitized data points just prior to and between 600 and 800 msec following onset of the pulse. Transepithelial and intracellular voltage data were acquired at a sampling rate of 10 Hz/channel on a Zenith 158 microcomputer (Zenith Data Systems, St. Joseph, MI). Using the "lumped" equivalent circuit shown in Fig. 1(*A*), the chord transepithelial resistance, R_t ($\Delta V_{ms}/I_t = [R_s(R_a + R_b)]/(R_a + R_b + R_s)$), and the apparent apical membrane fractional resistance, fR_a ($\Delta V_{mc}/\Delta V_{ms} = R_a/(R_a + R_b)$) were calculated from the changes in transepithelial and cell membrane voltages, appropriately corrected for series resistances. The effects of TEA^+, Ba^{2+}, and low pH on the control I_t-V relationships were assessed 30 to 60 sec after mucosal exposure of the epithe-

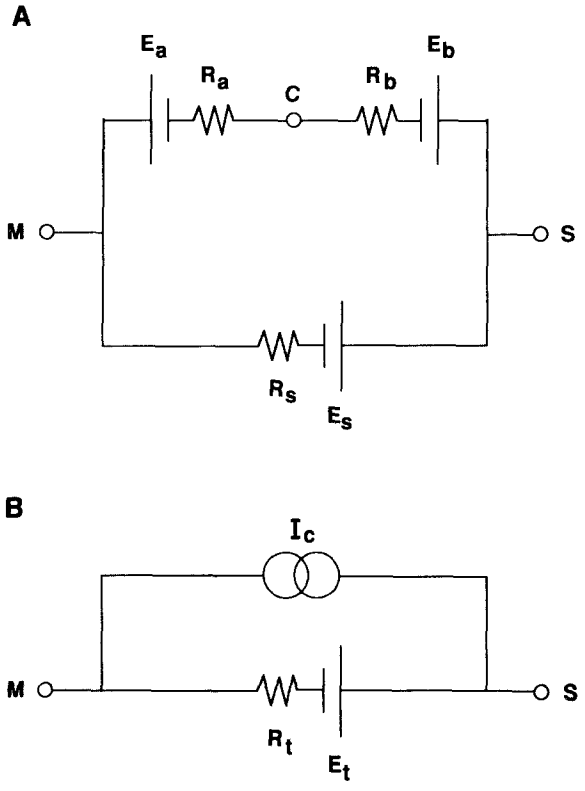

Fig. 1. Equivalent electrical circuits of *Necturus* gallbladder epithelium. *M*, *C* and *S* refer to mucosal, cellular and serosal compartments, respectively. (*A*) Equivalent "lumped" circuit model of the epithelium in which the cellular pathway is represented as the series arrangement of two Thévenin equivalents (apical and the basolateral membrane, respectively). Resistances are indicated by R_a and R_b, respectively, and zero-current voltages (i.e., equivalent emf's) by E_a and E_b, respectively. Although R_a is depicted as constant, we will show that it is voltage dependent. The paracellular (or shunt) pathway is also represented with a Thévenin equivalent (R_s and E_s). With the tissue bathed symmetrically with identical Ringer's solutions, E_s is assumed to be zero. (*B*) Reduction of the parallel arrangement of cellular and paracellular pathways to the simplest Thévenin equivalent circuit of the entire epithelium. The transepithelial resistance (R_t) is the parallel equivalent of R_s and ($R_a + R_b$). The transepithelial emf (E_t) is a function of all elements in the circuit. A current clamp (I_c) can be applied across the tissue

lium to these agents. Reversibility was determined by bracketing the I_t-V relationship obtained under these conditions with a second, control I_t-V study.

Long-Term Current Clamps

To assess the voltage- and time-dependence of the cell membrane conductances, long-term transepithelial constant-current studies were performed. The tissue was current-clamped for 1 min at ± 200 $\mu A/cm^2$ and brief (1 sec) constant-current pulses of ± 50 $\mu A/cm^2$ were superimposed, at 20-sec intervals, to measure fR_a and R_t. The brief current applications are referred to in the text as "pulses," in contrast to the 1-min "clamp." Both the voltage deflections produced by the pulses (before, during and after the clamp) and by the onset and the end of the current clamp itself were used to estimate fR_a and R_t (*see below*). To determine the relative contributions of R_a and R_b to the change in fR_a during the clamp, the cable properties of the epithelium were studied by means of intracellular current injection using a modification of the procedure described in detail elsewhere (Frömter, 1972; Reuss & Finn, 1975*a*). Intracellular current (~10 nA, 1-sec duration) was applied through one microelectrode and the resulting voltage deflection (ΔV_x) in a neighboring cell was measured. Ideally, ΔV_x is determined at several interelectrode distances x and from these data the parallel equivalent of apical and basolateral membrane resistances, $R_z = (R_a \cdot R_b/(R_a + R_b))$, can be determined. Since this procedure requires multiple cell impalements and several minutes to complete, a two-point cable analysis was done by measuring ΔV_x, at 20-sec intervals during continuous cell impalements, at a single distance x before, during and after the 1-min current clamp. The results are expressed as an "apparent" R_z ($R_z^{app} = \Delta V_x/\Delta V_x$ control, where ΔV_x is a voltage deflection at any time and ΔV_x control is the average of the three values prior to the clamp). The cable studies, together with the fR_a measurements, permitted qualitative inferences regarding changes in apical and basolateral membrane resistances. Two experimental protocols were used in these long-term current-clamp studies to estimate resistances. In the first, the transepithelial current pulses were applied in the m-to-s (i.e., V_{mc}-hyperpolarizing) direction, and the intracellular current pulses were also V_{mc}-hyperpolarizing. In the second, both the transepithelial and the intracellular current directions were reversed (i.e., both pulses were V_{mc}-depolarizing).

Analysis of V_{ms} Transients During 1-min Current Clamps

As described in detail previously (Reuss & Finn, 1977), the transient changes in V_{ms} in response to a 1-min constant-current clamp can be described using the simplified equivalent circuit shown in Fig. 1(*B*). The observed change in transepithelial voltage (ΔV_{ms}^{obs}) can in principle result from a change in transepithelial resistance (R_t), tissue equivalent emf (E_t), or both, and is described by the relation (Reuss & Finn, 1977):

$$\Delta V_{ms}^{obs} = \Delta V_{ms}^{R_t} + \Delta V_{ms}^{E_t}. \quad (1)$$

In practice, ΔR_t (i.e., the change in R_t during the clamp) can be determined either from the difference in: (1) the R_t values measured from the rising and falling V_{ms} deflections elicited by the 1-min current clamp itself, or (2) the R_t values measured from the voltage deflections caused by the pulses applied before, and during the 1-min clamp. The contribution of the change in R_t occurring during the clamp to the total change in V_{ms} is then:

$$\Delta V_{ms}^{R_t} = \Delta R_t I_t \quad (2)$$

provided that R_t and/or E_t do not change during the pulses themselves (or during the rising and falling phases of the 1-min current clamp). Although the two methods of estimating ΔR_t were expected to give the same result, a clear discrepancy was observed in the experiments with s-to-m clamps. For reasons detailed in Results and Discussion, we believe that the resistance estimates determined from the start and end of the current clamp are more accurate. These data were therefore utilized in the $\Delta V_{ms}^{R_t}$ calculations.

Statistics

Summary values are expressed as the mean ± SEM. Significant differences between groups were determined by conventional t-tests for paired data. A value of $P < 0.05$ was considered significant.

Results

Transepithelial and Cell Membrane I_t-V Relationships

The I_t-V_{ms} relationship measured with 1-sec transepithelial current pulses was nearly linear over the current range of ± 100 $\mu A/cm^2$ (Fig. 2*A*). At the lowest current density used in these studies (± 50 $\mu A/cm^2$), R_t was 156 ± 15 $\Omega \cdot cm^2$. With m-to-s pulses, R_t fell progressively as I_t was increased, to 148×13 $\Omega \cdot cm^2$ when $I_t = 450$ $\mu A/cm^2$. Conversely, R_t rose with s-to-m pulses, to 167 ± 17 $\Omega \cdot cm^2$ when $I_t = -450$ $\mu A/cm^2$. The changes in R_t are larger with 1-min current clamps and can be attributed to changes in the paracellular pathway, as will be discussed below.

The concomitant changes in cell membrane voltages are plotted against V_{ms} in Fig. 2(*B*). With m-to-s currents, V_{mc} hyperpolarized and V_{cs} depolarized in a near-linear manner. In contrast, clear deviations from linearity were observed with s-to-m currents, which are due to a decrease in fR_a (*see* Fig. 2*C*), in agreement with the observations of García-Díaz et al. (1983). Current application in the m-to-s direction (i.e., to positive values of V_{ms}) resulted in small increases in fR_a, as exemplified in Fig. 2(*C*). Conversely, a marked decrease in fR_a was observed when s-to-m pulses were applied. The voltage dependence of fR_a was most pronounced in the V_{ms} range of -20 to -60 mV (Fig. 2*C*).

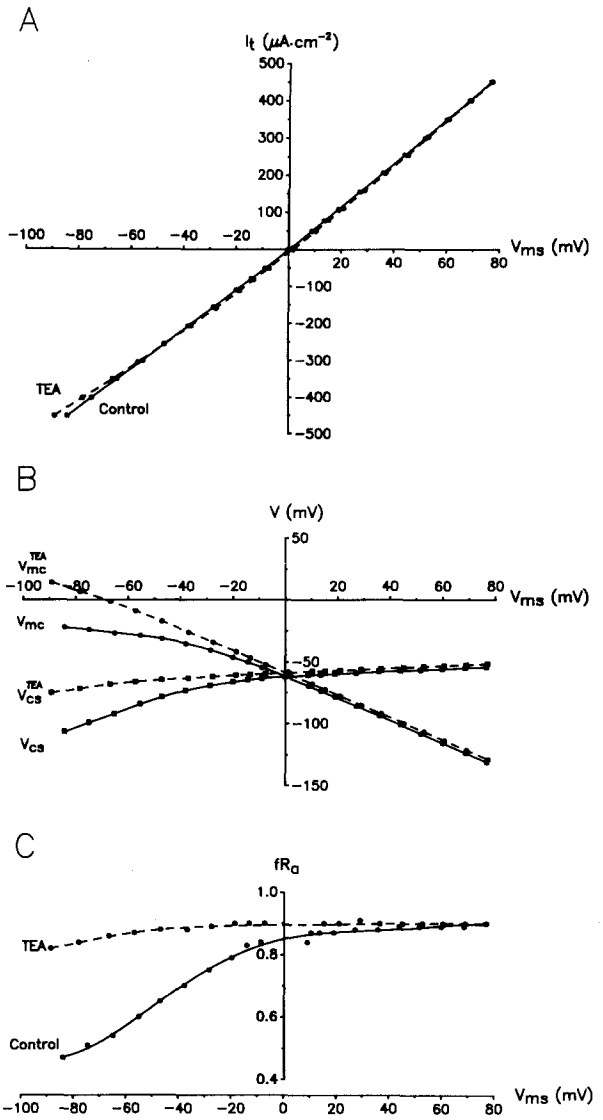

Fig. 2. Effects of transepithelial current pulses (1-sec duration) on transepithelial voltage (*A*), cell membrane voltages (*B*) and apical membrane fractional resistance (*C*). The results shown are from a single representative experiment. Control data (no TEA^+): open symbols connected by solid lines. Experimental data (5 mM TEA^+): solid symbols connected by dashed lines. Values have been corrected for solution series resistances. Note that TEA^+ has little effect on the I_t-V_{ms} relationship (panel *A*), but reduces sizably the effects of the *s*-to-*m* current pulses on fR_a (panel *C*). In the presence of TEA^+, the relationships between V_{mc} and V_{cs} and the current-induced transepithelial voltage become nearly linear (panel *B*)

Effect of K^+ Channel Inhibitors on I_t-V Relationships

We tested the effects of TEA^+, Ba^{2+} and external pH on the I_t-V relationships. As illustrated in Fig. 2(*A*), in the presence of 5 mM TEA^+ in the mucosal solution only at large *s*-to-*m* currents did the slope of the I_t-V_{ms} relationship deviate significantly from control. This is likely due to an increase in paracellular current flow because TEA^+ blocks the voltage-dependent decrease in fR_a (Fig. 2*C*). As depicted in Fig. 2(*B*) and 2(*C*), 5 mM TEA^+ caused partial linearization of both the V_{mc}-V_{ms} and fR_a-V_{ms} relationships. In the presence of TEA^+, fR_a remained essentially constant over the V_{ms} range of +80 to −50 mV, decreasing when $V_{ms} > -50$ mV, which corresponds to a depolarization of V_{mc} of greater than 40 mV. Current-voltage relationships were also determined in the presence of Ba^{2+} (5 mM) and during acidification of the mucosal solution (by 1 unit, to pH 6.65). Calculated values of fR_a under control conditions or during exposure to TEA^+, Ba^{2+} or acidification, are plotted against V_{mc} in Fig. 3. The control relationship between fR_a and V_{mc} appeared sigmoidal. Mucosal addition of either TEA^+ or Ba^{2+} reduced the voltage-dependent decrease in fR_a, confirming the results of García-Díaz et al. (1983). Ba^{2+} was less potent than TEA^+, especially when V_{mc} was depolarized below −40 mV. Acidification of the mucosal solution had a much smaller effect. Because of the presence of apical Na^+/H^+ and Cl^-/HCO_3^- exchangers, when mucosal [HCO_3^-] is lowered the cells acidify by approximately 0.15 units (Stoddard & Reuss, 1985; Reuss, Costantin & Bazile, 1987). Hence, neither extracellular nor intracellular acidification has major effects on the voltage dependence of fR_a, at least over these pH ranges. García-Díaz et al. (1983) demonstrated that mucosal solution acidification to pH 5.5 largely reduced the voltage-dependent decrease in fR_a. These authors also found that serosal TEA^+ had no effect on the voltage-induced change in fR_a. We confirmed this finding in two experiments (*data not shown*).

Whereas Ba^{2+} had a small depolarizing effect on the baseline ($I_t = 0$) cell membrane voltages, TEA^+ had no significant effect (Table 1), although both cations significantly increased fR_a and reduced its voltage dependence. Since the apical membrane is K^+ selective (van Os & Slegers, 1975; Reuss & Finn, 1975*b*), one possible interpretation of these results is the coexistence of two apical K^+ channels, which differ in kinetics and sensitivity to inhibitors (*see* Discussion). In contrast with the effects of TEA^+ and Ba^{2+}, mucosal solution acidification caused no significant change in fR_a. The small mucosa-positive increase in V_{ms} with TEA^+ (Table 1) is most likely due to a junctional Na^+/TEA^+ bi-ionic potential.

Representative records of the time courses of voltages and fR_a during transepithelial current pulses are shown in Fig. 4. Within this time frame,

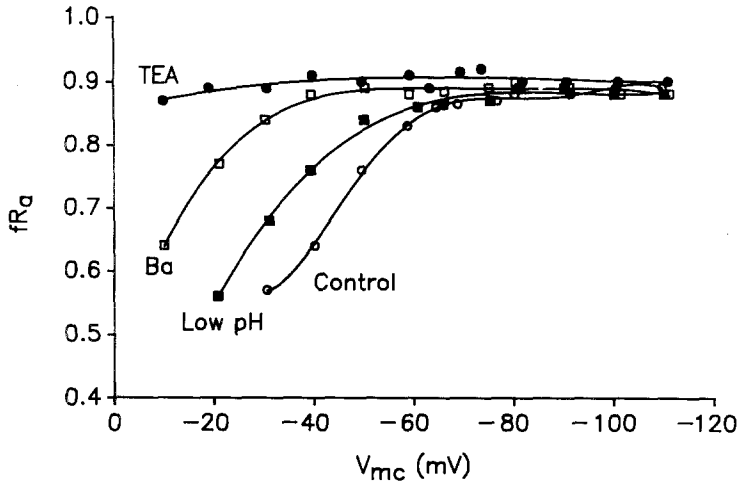

Fig. 3. Relationship between fR_a and V_{mc} in the absence and presence of K^+ channel inhibitors. Changes in V_{mc} were elicited by transepithelial current pulses of 1-sec duration. Data shown are means of six experiments. The effects of TEA^+ (5 mM), Ba^{2+} (5 mM), and low pH (6.65) were tested in each preparation. Standard errors ranged from 0.01 to 0.08 and from <1 to 3 mV, for fR_a and V_{mc}, respectively. Symbols: control: open circles, TEA^+: filled circles, Ba^{2+}: open squares, low pH: filled squares. For baseline values in the absence of transepithelial current application, *see* Table 1

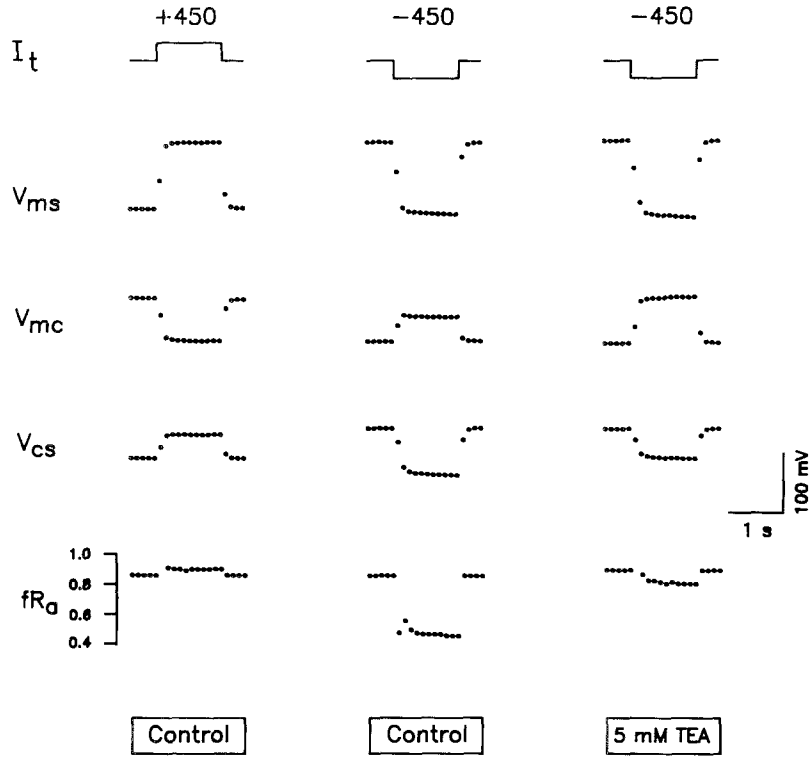

Fig. 4. Selected records of digitized voltage (uncorrected for solution series resistances) and fR_a values from the experiment depicted in Fig. 2. Current polarity and density indicated at the top (current density in $\mu A/cm^2$; *m*-to-*s* current positive). The records on the right-hand column were obtained in the presence of 5 mM TEA^+ in the mucosal solution. Note that fR_a attains a pseudo-steady value within 500 msec and that TEA^+ markedly reduced the voltage-dependent change in fR_a observed during the serosa-to-mucosa current pulse

the voltages and fR_a appear to reach steady values within 500 msec. However, we will show that during *s*-to-*m* current clamps there is a second, slow decrease in fR_a with a half-time of seconds. Preexposure of the epithelium to TEA^+ reduced significantly the change in fR_a at -450 $\mu A/cm^2$ with no apparent effect on the time course of the voltage transients. Similar results were obtained at lower current densities (*data not shown*). Although the low sampling rate in these studies precluded an accurate measurement of the time constant of the voltage responses, an upper limit of about 100 msec can be estimated, which is similar to that of the capacitive transients measured in *Necturus* gallbladder by others (García-Díaz et al., 1983; García-Díaz & Essig, 1985). Therefore, the voltage-dependent decrease in fR_a is rapid, with a $t_{1/2}$ similar to or less than that of capacitive transients.

Table 1. Changes in the electrical parameters of *Necturus* gallbladder upon mucosal exposure to TEA^+, Ba^{2+} or low pH[a]

	ΔV_{ms} (mV)	ΔV_{mc} (mV)	ΔV_{cs} (mV)	ΔfR_a	ΔR_t ($\Omega \cdot cm^2$)
TEA^+	0.9 ± 0.2*	−0.4 ± 0.6	0.5 ± 0.5	0.03 ± 0.01*	−1 ± 2
Ba^{2+}	0.9 ± 0.1*	2.4 ± 0.7*	3.4 ± 0.7*	0.02 ± 0.01*	14 ± 3*
Low pH	−0.3 ± 0.1*	2.5 ± 0.8*	2.3 ± 0.8*	−0.01 ± 0.01	3 ± 3

[a] Values are means ± SEM of $n = 6$ experiments. Changes are expressed as experimental-control. Control values in these studies were: $V_{ms} = 0.5 \pm 0.1$ mV; $V_{mc} = -69 \pm 2$ mV; $V_{cs} = -68 \pm 2$ mV; $fR_a = 0.87 \pm 0.01$; $R_t = 156 \pm 15\ \Omega \cdot cm^2$. *$P < 0.05$.

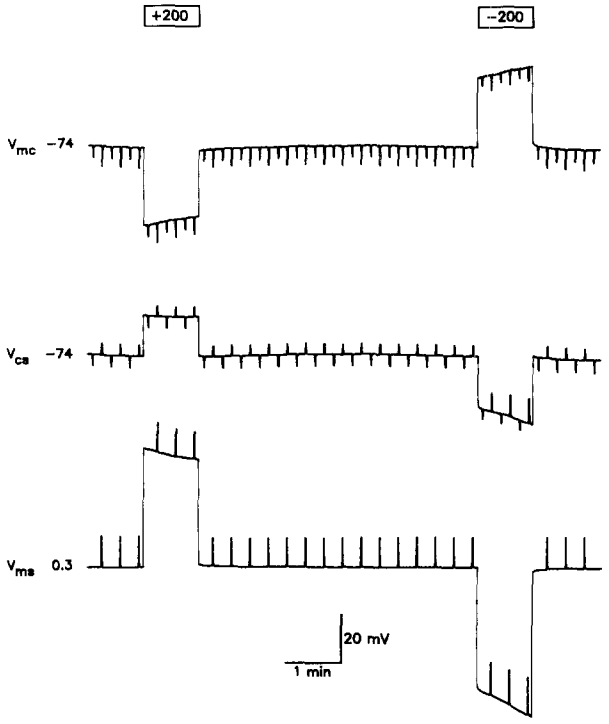

Fig. 5. Changes in transepithelial and cell membrane voltages in response to 1-min mucosa-to-serosa (left) and serosa-to-mucosa (right) current clamps (200 $\mu A/cm^2$). The brief upward deflections in the V_{ms} and V_{cs} traces were produced by transepithelial current pulses (1-sec duration, 20-sec interval, 50 $\mu A/cm^2$) applied to measure fR_a and R_t. The brief downward deflections in the V_{cs} trace (ΔV_x) were in response to intracellular current pulses applied in a neighboring cell; R_z^{app} was calculated from these deflections. This experiment is an example of a V_{mc}-hyperpolarizing pulse protocol (*see* Materials and Methods). Note that during the −200 μA clamp fR_a fell and R_z^{app} was less than 1, whereas R_t increased

Effects of 1-min Mucosa-to-Serosa Current Clamps on Voltages and Resistances

To determine which membrane(s) contribute to voltage- and/or time-dependent changes in fR_a, tissues were current-clamped for 1-min periods at ±200 $\mu A/cm^2$ and the cable properties of the epithelium were concurrently determined. The changes in membrane voltages and resistances which occur within 600 msec are hereafter referred to as the "early" events, whereas those measured from 600 msec after the onset of the clamp to just prior to its end will be referred to as the "late" events. A representative record of a 1-min *m*-to-*s* current clamp is shown in Fig. 5 (left). Following the early voltage deflections, the transepithelial and cell membrane voltages return slowly towards pre-clamp values (*see* Fig. 5 and Table 2). Immediately following termination of the clamp, the transepithelial voltage remained mucosa positive by 1.5 mV ($\Delta V_{ms}^{E_t}$, Table 2), and gradually returned to control over the next several minutes.

Resistance data obtained in seven tissues are summarized in Fig. 6 (panels *A–C*). The fR_a and R_t values were estimated from the deflections elicited by the current clamp (onset and end, symbols joined by solid lines during the clamp) and by superimposed 1-sec pulses (*see* Materials and Methods). For reasons that will become apparent below, linear changes with time were assumed for both fR_a and R_t during the current clamp. The fall in R_t during the clamp was gradual and followed the time course of the late change in V_{ms} (Fig. 6*C*), taking several minutes to return to control values after the end of the clamp. Although the estimates of R_t from the pulses were lower than the values predicted assuming a linear change in R_t with time, the differences were small.

The late decrease in R_t during the clamp cannot be due to changes in cell membrane resistances because, although fR_a rose immediately after the onset of the clamp, it remained constant thereafter. In addition, R_z^{app} remained unchanged throughout the duration of the clamp.[1] These results suggest that

[1] In theory, an $R_z^{app} > 1$ would have been predicted from a voltage-dependent increase in R_a. However, because $R_a \gg R_b$ (Stoddard & Reuss, 1988) and the parallel arrangement $R_z = R_aR_b/(R_a + R_b)$ (*see* Materials and Methods), the change in ΔV_x could be small enough to be undetectable, so that R_z^{app} would remain equal to 1. In contrast, if the increase in fR_a were due to a decrease in R_b, R_z^{app} would have been reduced below unity.

Table 2. Late changes in membrane voltages during 1-min current clamps and estimation of $\Delta V_{ms}^{R_t}$ and $\Delta V_{ms}^{E_t}$ (V_{mc}-hyperpolarizing pulses)[a]

Current-clamp direction	ΔV_{mc} (mV)	ΔV_{cs} (mV)	ΔV_{ms} (mV)	$\Delta V_{ms}^{R_t}$ (mV)	$\Delta V_{ms}^{E_t}$ (mV)
m to *s*	2.5 ± 0.7	−0.7 ± 0.3	−3.3 ± 0.6	−4.8 ± 0.6	1.5 ± 0.1
s to *m*	2.4 ± 0.6	−5.6 ± 1.0	−8.0 ± 1.2	6.0 ± 1.2	−2.0 ± 0.1

[a] Values are means ± SEM of $n = 7$ experiments. Current-clamp density was 200 $\mu A/cm^2$. $\Delta V_{ms} \equiv \Delta V_{ms}^{obs}$. Polarities of $\Delta V_{ms}^{R_t}$ and $\Delta V_{ms}^{E_t}$ are defined as follows: a fall in R_t causes a negative $\Delta V_{ms}^{R_t}$, and a mucosa-negative change in E_t yields a negative change in $\Delta V_{ms}^{E_t}$. *See* Materials and Methods for additional details of measurements of $\Delta V_{ms}^{R_t}$ and $\Delta V_{ms}^{E_t}$.

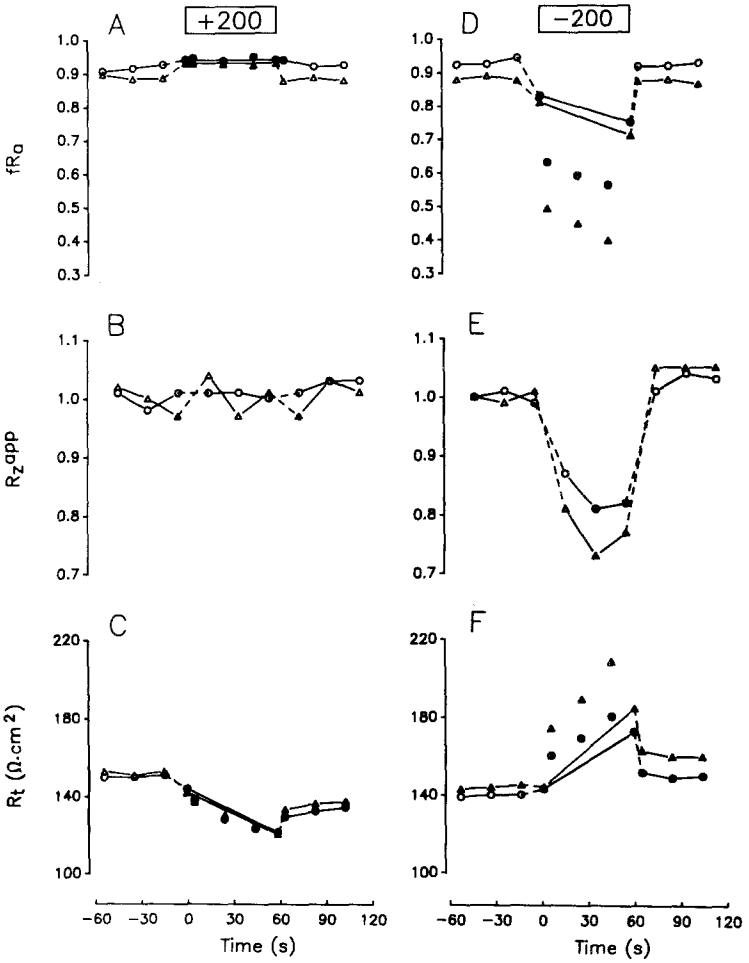

Fig. 6. Changes in fR_a, R_z^{app} and R_t during mucosa-to-serosa current clamps (left, panels *A*, *B* and *C*, respectively) and serosa-to-mucosa current clamps (right, panels *D*, *E* and *F*, respectively). Two sets of data are shown in each panel. The symbols denote V_{mc}-hyperpolarizing and V_{mc}-depolarizing pulse protocols (circles and triangles, respectively). Filled symbols indicate statistically significant changes compared to control (control = mean of the three values preceding the current clamp). The solid lines before and after the clamps join control data. The solid lines during the clamp (in fR_a and R_t panels) join data calculated from the voltage deflections produced by the onset and end of the clamp itself. The dashed lines join these values with the pre-clamp and post-clamp controls. $n = 7$ experiments. Standards errors have been omitted for clarity. Note that one of the fR_a values during the clamp in panel *A* was not significantly different from control

during *m*-to-*s* clamps there are no sizable late changes in R_a or R_b. These results also argue against possible late changes in E_a or E_b. Inasmuch as the late fall in R_t caused by *m*-to-*s* clamps cannot be due to changes in cell membrane resistances, it must be caused by a decrease in paracellular resistance (R_s).

The change in V_{ms} during the clamp can be due to changes in R_t and/or E_t (*see* Fig. 1*B*). From R_t values calculated from the V_{ms} deflections at the start and end of the clamp, the change in V_{ms} attributable to R_t, $\Delta V_{ms}^{R_t}$, was calculated to be ca. −5 mV (Table 2), which is greater than the change in V_{ms} actually observed. The difference (= $\Delta V_{ms}^{E_t}$) is due to a mucosa-positive change in E_t, opposite in polarity to the $\Delta V_{ms}^{R_t}$. The decrease in R_t during *m*-to-*s* clamps is the result of a fall in R_s (*see above*), attributed by several investigators to increases in either fluid conductivity or width of the lateral intercellular spaces (Frömter, 1972; Bindslev, Tormey & Wright, 1974; Reuss & Finn, 1977). The observed $\Delta V_{ms}^{E_t}$ has been attributed to a mucosa-positive increase in E_s, i.e., a junctional "polarization poten-

Table 3. Late changes in membrane voltages during 1-min current clamps and estimation of $\Delta V_{ms}^{R_t}$ and $\Delta V_{ms}^{E_t}$ (V_{mc}-depolarizing pulses)[a]

Current-clamp direction	ΔV_{mc} (mV)	ΔV_{cs} (mV)	ΔV_{ms} (mV)	$\Delta V_{ms}^{R_t}$ (mV)	$\Delta V_{ms}^{E_t}$ (mV)
m to *s*	2.3 ± 0.3	−0.6 ± 0.2	−2.8 ± 0.5	−4.4 ± 0.5	1.7 ± 0.1
s to *m*	3.4 ± 1.0	−6.6 ± 1.1	−10.1 ± 1.7	8.0 ± 1.8	−2.0 ± 0.1

[a] Values are means ± SEM of $n = 7$ experiments. Current-clamp density was 200 $\mu A/cm^2$. $\Delta V_{ms} \equiv \Delta V_{ms}^{obs}$. For polarities of $\Delta V_{ms}^{R_t}$ and $\Delta V_{ms}^{E_t}$, *see* Table 2. *See* Materials and Methods for additional details of measurement of $\Delta V_{ms}^{R_t}$ and $\Delta V_{ms}^{E_t}$.

tial" (Wedner & Diamond, 1969; Bindslev et al., 1974; Reuss & Finn, 1977) due to the transport-number effect (*see* Barry & Hope, 1969*a,b*).

The decrease in R_s during the clamp reduces the fraction of I_t passing through the cells, causing in turn a late depolarization of V_{mc}, of relative magnitude determined by fR_a (*see* Fig. 1). Concomitantly, the mucosa-positive change in E_s would be expected to hyperpolarize V_{mc}. Since $\Delta V_{ms}^{R_t} > \Delta V_{ms}^{E_t}$, the decrease in R_s dominates in causing the changes in V_{mc} and V_{cs}. Inasmuch as fR_a was high averaging 0.94, $\Delta V_{mc} > \Delta V_{cs}$ (*see* Table 2). Although small changes in E_a or E_b may also occur, the late changes in cell membrane voltage during *m*-to-*s* current clamps are largely the result of late changes in R_s.

Effects of 1-min Serosa-to-Mucosa Current Clamps on Voltages and Resistances

The transepithelial and cell membrane voltage changes elicited by a serosa-to-mucosa current clamp show characteristic late voltage transients, as shown in Fig. 5 (right). In each case, the late transients are in the same direction as the initial voltage deflections. In addition, the late changes in V_{ms} and V_{cs} were larger than those seen with *m*-to-*s* clamps (Table 2). Calculations similar to those described above indicate that the late increase in V_{ms} is due to an elevation of R_t in combination with a mucosa negative change in E_t (E_s). The R_t effect is dominant. The observed changes in cell membrane voltages result in part from these paracellular alterations, but in addition there is a late fall in fR_a (Fig. 6*D*), although a discrepancy in fR_a values was found when comparing pulses *vs.* linear interpolation of clamp estimates (*see below*).

The decrease in fR_a during the *s*-to-*m* current clamp could result in principle from a decrease of R_a, an increase of R_b, or a combination of effects. The observed fall in R_z^{app} (Fig. 6*E*) rules out an increase in R_b alone and suggests instead a fall in R_a. The lack of a decrease at the first measurement of R_z^{app} during the current clamp can be explained by the direction of the intracellular current. Because the voltage-dependent changes in R_a occur within 500 msec (*see above*) and since the intracellular current hyperpolarized V_{mc}, R_a increases during the measurement itself, offsetting the expected decrease in R_a, and hence in R_z^{app}, induced by the transepithelial current clamp. This effect should be most manifest at small interelectrode distances (x) because of the rapid attenuation of ΔV_x with distance (Frömter, 1972; Reuss & Finn, 1975*a*; Stoddard & Reuss, 1988). In agreement with this interpretation, when $x = 53 \pm 7$ μm R_z^{app} early in the clamp averaged 0.98×0.07 ($n = 3$) whereas for $x = 123 \pm 16$ μm R_z^{app} decreased to 0.79 ± 0.06 ($n = 4$). In addition, with V_{mc}-depolarizing pulses (triangles in Fig. 6*E*) all values of R_z^{app} during the clamp were significantly lower than 1. In conclusion, the decrease in fR_a during *s*-to-*m* clamps is largely due to a fall in R_a. Inasmuch as the clamp depolarizes V_{mc}, the simplest explanation of this result is a voltage-dependent K^+ conductance of the apical membrane (*see* Discussion).

Resistance Measurements: Dependence on Direction of Applied Current

The studies described above indicate that the resistance estimates obtained from superimposed pulses during the 1-min *s*-to-*m* clamp are compromised by the voltage-dependent increase in apical membrane conductance which takes place during the measurement itself. To test this idea further, in the same tissues we reversed the direction of both the transepithelial and intracellular current pulses. The late changes in transepithelial and cell membrane voltages, summarized in Table 3 (V_{mc}-depolarizing pulses), were similar to those obtained in the studies summarized in Table 2 (V_{mc}-hyperpolarizing pulses), as expected given the identity of the 1-min transepithelial current clamps. However, important differences were noted in the resistance estimates.

The pre-clamp control values of fR_a in both *m*-to-*s* and *s*-to-*m* studies were lower with V_{mc}-de-

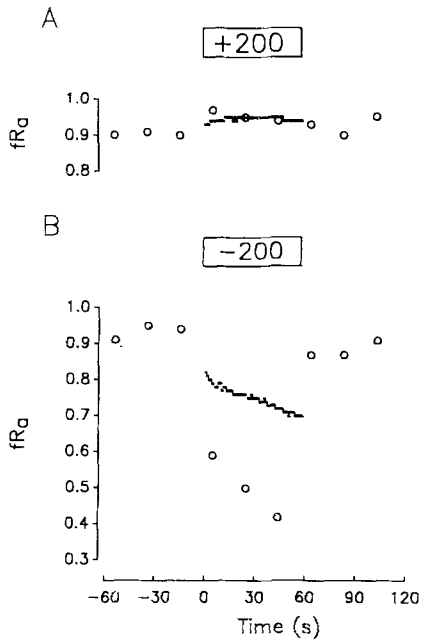

Fig. 7. Time course of fR_a during 1-min mucosa-to serosa (panel *A*) and serosa-to-mucosa (panel *B*) current clamps (200 μA/cm^2). The results were calculated from the data of the experiment shown in Fig. 5. The large open circles correspond to calculations of fR_a from 1-sec pulses. The small filled circles plotted during the current clamps are the fR_a values calculated from the digitized V_{ms} and V_{cs} records, with appropriate corrections for series resistances, starting 600 msec after onset of the clamp, and continuing at 1-sec intervals for the duration of the clamp. Note that the late change in fR_a during the -200 μA clamp is approximately linear with time

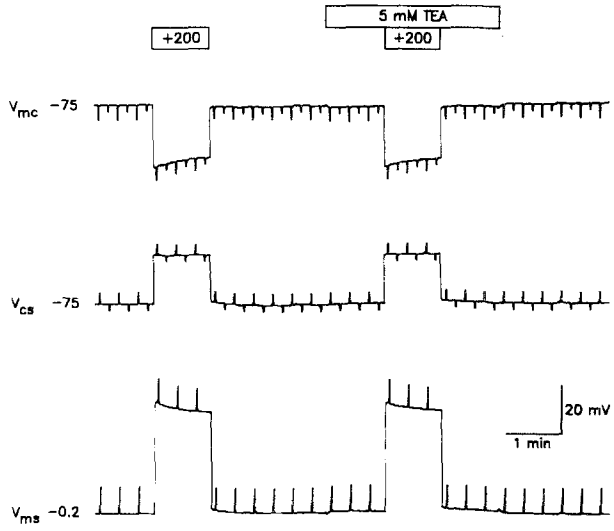

Fig. 8. Changes in transepithelial and cell membrane voltages in response to 1-min mucosa-to-serosa current clamps (200 μA/cm^2), under control conditions (left) and in the presence of 5 mM TEA^+ in the mucosal solution (right). Format as in Fig. 5

polarizing pulses (Fig. 6*A* and 6*D*). The difference was small but significant, demonstrating that a finite voltage sensitivity of fR_a exists near control values of V_{mc}, as was observed in the I_t-V studies described above.

During *m*-to-*s* clamps, fR_a was significantly increased in all three measurements using V_{mc} depolarizing pulses and in two of three using V_{mc}-hyperpolarizing pulses (Fig. 6*A*). Importantly, these estimates of fR_a (i.e., from superimposed pulses) were similar to the assumed linear change of fR_a with time during the clamp (i.e., interpolated between the fR_a estimates obtained from the start and end of the clamp). This result is expected because in these studies V_{mc} was hyperpolarized by ~27 mV during the clamp, and hence in a region of the fR_a-V_{mc} relationship where fR_a is voltage-independent (Fig. 3). For the same reason, no significant change in R_z^{app} was measured (Fig. 6*B*) regardless of the polarity of the intracellular pulses. We also obtained similar estimates of R_t with both transepithelial polarities (Fig. 6*C*).

In contrast to the *m*-to-*s* studies, during *s*-to-*m* clamps both V_{mc}-depolarizing and V_{mc}-hyperpolarizing pulses yielded large underestimates of fR_a compared with calculations based on the voltage deflections at the onset and end of the clamp (Fig. 6*D*). These results are consistent with the notion that the voltage-dependent changes in R_a are rapid (<500 msec) and hence that R_a changes during the measurement period itself (*see* Discussion).

To examine in greater detail the time course of the change in fR_a during *m*-to-*s* and *s*-to-*m* clamps, the values of fR_a calculated from the clamp-induced voltage deflections of the study shown in Fig. 5 are plotted in an expanded format in Fig. 7. Also shown are the fR_a estimates obtained from the superimposed pulses. At the onset of the *m*-to-*s* clamp fR_a increased slightly (Fig. 7*A*) and remained essentially constant throughout the duration of the clamp. In contrast, during the *s*-to-*m* clamp fR_a decreased rapidly at the onset of the clamp and continued to decline at a slower, near-linear rate while the current was maintained. This observation justifies the interpolation of fR_a values described above.

Effect of TEA^+ on 1-min Current Clamps

Representative records depicting the effect of 5 mM TEA^+ on *m*-to-*s* current clamps are shown in Fig. 8. Addition of TEA^+ to the mucosal solution caused a small mucosa-positive increase in V_{ms} with no significant changes in baseline cell membrane voltages. The secondary changes in V_{ms} and V_{mc} were smaller with TEA^+ (Table 4) because of a lower $\Delta V_{ms}^{R_t}$ which

Table 4. Late changes in membrane voltages and estimation of $\Delta V_{ms}^{R_t}$ and $\Delta V_{ms}^{E_t}$ during 1-min current clamps in the absence and presence of 5 mM TEA^+ in the mucosal solution[a]

Condition	Control	5 mM TEA^+	Control	5 mM TEA^+
Current-clamp direction	*m* to *s*	*m* to *s*	*s* to *m*	*s* to *m*
ΔV_{mc}	3.0 ± 0.5	2.0 ± 0.4	8.6 ± 0.8	26.9 ± 3.2*
ΔV_{cs}	−1.0 ± 0.5	−1.0 ± 0.4	−11.3 ± 2.2	−6.3 ± 3.2*
ΔV_{ms}	−3.9 ± 0.3	−2.9 ± 0.2*	−19.8 ± 2.8	−33.2 ± 5.8*
$\Delta V_{ms}^{R_t}$	−5.7 ± 0.4	−4.6 ± 0.3*	17.8 ± 2.6	30.8 ± 5.5*
$\Delta V_{ms}^{E_t}$	1.8 ± 0.2	1.6 ± 0.2	−2.1 ± 0.2	−2.6 ± 0.4*

[a] Values are means ± SEM, in mV for $n = 6$ experiments for all groups. Current-clamp density was 200 $\mu A/cm^2$. *$P < 0.05$, compared to control. For polarity conventions, *see* Table 2.

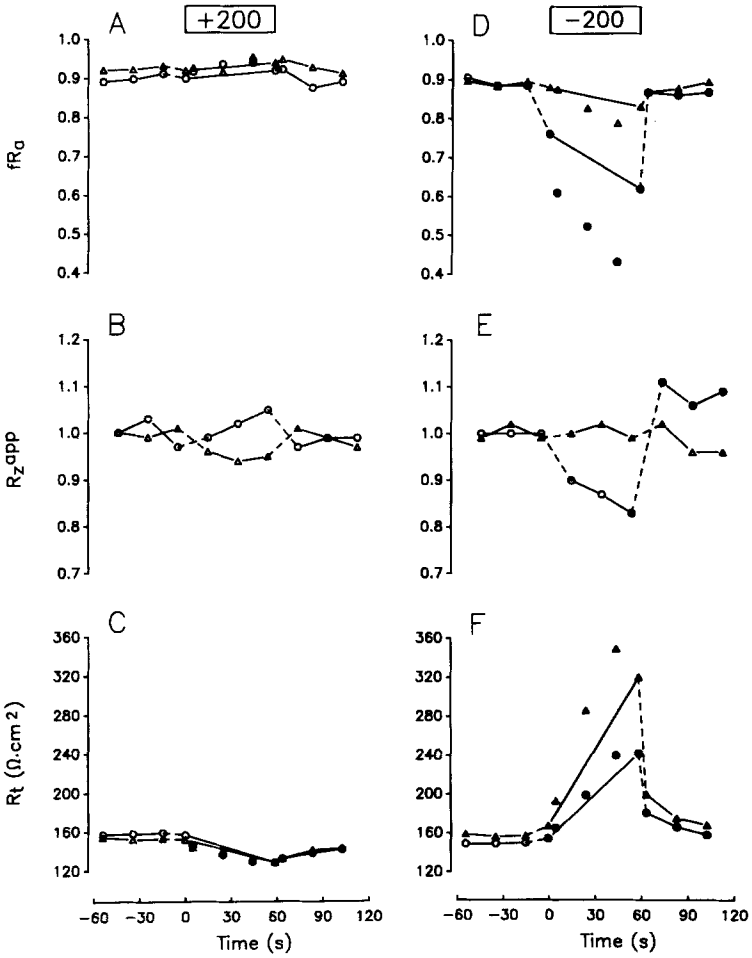

Fig. 9. Changes in fR_a, R_z^{app} and R_t during mucosa-to-serosa current clamps (left, panels *A*, *B* and *C*) and serosa-to-mucosa current clamps (right, panels *D*, *E* and *F*). Format as in Fig. 6. Symbols denote absence or presence of 5 mM TEA^+ in the mucosal solution (circles and triangles, respectively). Solid symbols indicate statistically significant changes compared to the mean of the three pre-clamp values. Resistances were measured using V_{mc}-hyperpolarizing pulses. $n = 6$ experiments

is most likely due to incomplete recovery of R_t from the preceding control current clamp (Fig. 9*C*). In these tissues, in the absence of TEA^+, a voltage-dependent increase in fR_a in response to the clamp was observed in only three of six experiments (Fig. 9*A*), whereas in another series (Fig. 6*A*) a small, but significant increase in fR_a was observed. Hence, the change in fR_a during *m*-to-*s* clamps is either small or absent, thus making the test for an effect of TEA^+ impossible. Therefore, no significant changes in fR_a (Fig. 9*A*) or in cable properties (R_z^{app} in Fig. 9*B*) were observed during the *m*-to-*s* clamp when the tissue was exposed to TEA^+.

Representative traces of *s*-to-*m* current clamps

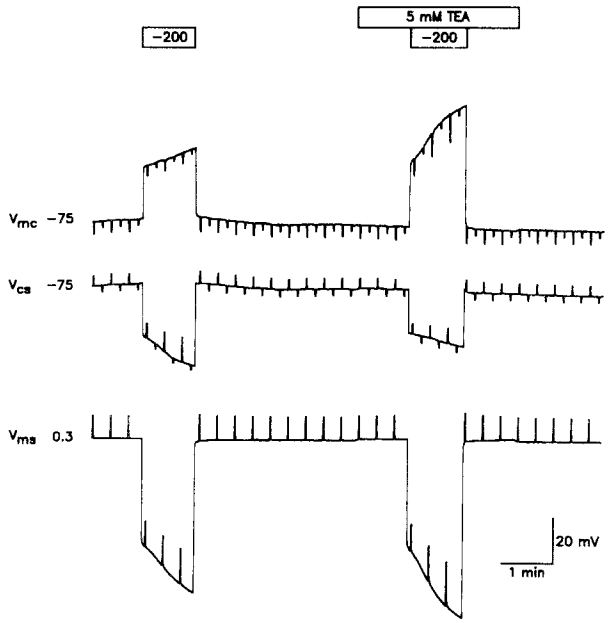

Fig. 10. Changes in transepithelial and cell membrane voltages in response to 1-min serosa-to-mucosa current clamps (−200 $\mu A/cm^2$), under control conditions (left) and in the presence of 5 mM TEA^+ in the mucosal solution (right). Format as in Fig. 5. Note that TEA^+ abolished the decrease in ΔV_x and markedly reduced the decrease in fR_a in response to the current clamp

obtained in the absence and presence of TEA^+ are shown in Fig. 10. The results of these studies are summarized in Fig. 9 (panels *D-F*) and Table 4. The control data in this series of experiments differed in several respects from the results shown in Fig. 5 and Tables 2 and 3. In particular, after the end of the clamp R_z^{app} was elevated, whereas fR_a was significantly below control.[2] These results indicate that R_b increased during the clamp, contributing to the fall in fR_a and accounting, at least in part, for the initial constancy of R_z^{app}. Because the basolateral membrane is mainly K^+ permeable (Reuss, 1979; Stoddard & Reuss, 1988), the increase in R_b could be due to depletion of K^+ in the fluid layer adjacent to the basolateral membrane resulting in a decrease in g_K (Reuss & Finn, 1977). Alternatively, the elevation of R_b could be due to a decrease in basolateral P_K.

Addition of TEA^+ to the mucosal solution had small effects on transepithelial and cell membrane voltages, but the decrease in fR_a and the changes in cable properties produced by the clamp were abolished (*see* Figs. 9 and 10). Finally, we note that the values of R_t estimated from pulse data during the clamp were consistently higher than those predicted from interpolation between the V_{ms} deflections at the onset and end of the clamp. The deviation was greater in the presence of TEA^+ than in its absence.

[2] Most of the differences between the control values in these two groups of tissues can be ascribed to a higher spontaneous R_s in the preparations used in the TEA^+ series. This difference would be expected to have effects on the magnitude of the cell membrane IR drops and the polarization effects resulting from the *s*-to-*m* current clamp.

Discussion

The results of the I_t-V experiments demonstrate that the voltage-induced changes in fR_a are sensitive to inhibitors of voltage-dependent K^+ channels found in epithelia (Hunter et al., 1986; Bolívar & Cereijido, 1987; Frindt & Palmer, 1987; Guggino et al., 1987). Of the agents tested, we found TEA^+ to be the most effective in preventing changes in fR_a. Its blocking action was essentially complete over the V_{mc} range of −30 to −110 mV (Fig. 3). The main argument for the conclusion that the voltage-dependent changes in fR_a are due primarily to changes in R_a is that during *s*-to-*m* current clamps both fR_a and R_z^{app} decreased. In addition, TEA^+ abolished the effects of the *s*-to-*m* clamp and was effective only from the apical surface.

Our results are in agreement with the suggestion of a voltage-dependent apical membrane conductance in *Necturus* gallbladder epithelium (García-Díaz et al., 1983), and in guinea-pig gallbladder (Gunter-Smith, 1987). In the present studies we employed a combination of two-point cable analysis, and R_t and fR_a measurements, which allowed for a more direct demonstration of the apical membrane location of the change in fR_a first reported by García-Díaz et al. (1983).

Time-Varying Changes in fR_a During Long-Term Current Clamps

The response of fR_a to a 1-min *s*-to-*m* current clamp had two distinct phases, the first occurring within 500 msec from the onset of the clamp with a half-time of ≤100 msec, and the second having a half-time of several seconds (*see* Fig. 7). The late decrease in fR_a can be explained in part by the late depolarization of V_{mc}. This depolarization is the result of the current-induced increase in paracellular resistance, which in turn causes an increase in transcellular current flow. Since the slope of the fR_a-V_{mc} relationship (in the V_{mc} range of −40 to −60) was ~0.1/10 mV (Fig. 3), and the late fall in V_{mc} averaged 8.6 mV (Table 4), we estimate that ca. 60% of the late fall in fR_a is due to the late depolar-

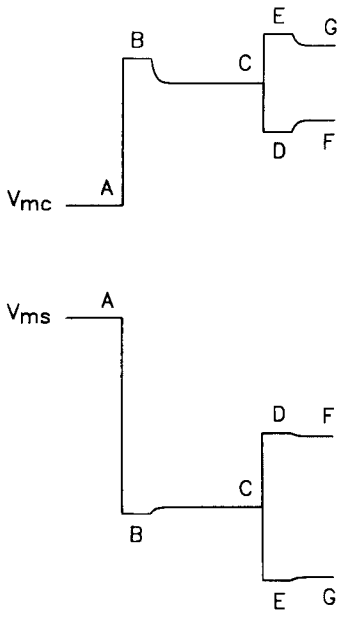

Fig. 11. Schematic illustration of the predicted changes in V_{mc} and V_{ms} in response to a transepithelial serosa-to-mucosa current clamp. *See* text for details

ization of the apical membrane. At the end of the *s*-to-*m* clamp, R_b was increased, contributing to the fall in fR_a. It is possible that alterations in intracellular pH and/or Ca^{2+} activity contribute to the time-varying changes in fR_a, for instance by shifting the voltage dependence of R_a (García-Díaz et al., 1983).

In a previous study, Reuss and Finn (1977) found no change in fR_a during similar *s*-to-*m* current clamps. This result was obtained because resistance estimates were made within 20 msec from the start of the clamp (or the superimposed pulse), i.e., at a time when capacitive transients are still contributing to V_{mc} (García-Díaz & Essig, 1985). However, we refer the reader to this report (*see* Figs. 5 and 6, Reuss & Finn, 1977) for a more detailed analysis of the "voltage transients" elicited by 1-sec pulses superimposed on the clamp, which can now be interpreted in terms of the half-time of the voltage-dependent change in R_a being $\leq$100 msec. Other investigators (García-Díaz et al., 1983; García-Díaz & Essig, 1985) have indicated that the half-time of the voltage-dependent change in R_a is of the order of seconds, based on fR_a measurements obtained at 3-sec intervals from pulses superimposed on the holding voltage. We believe their values of fR_a, as well as ours when determined from superimposed pulses, to be underestimates which yield a misleading time course of the change in fR_a.

To clarify this point, in Fig. 11 we depict the relative changes in V_{mc} and V_{ms} in response to a transepithelial *s*-to-*m* current clamp, under the simplifying restriction that the only equivalent circuit parameter allowed to change is R_a (*see* Fig. 1). The deflections A-B represent the instantaneous changes in V_{mc} and V_{ms} in response to the current clamp, at a constant value of R_a. A voltage-dependent decrease in R_a would cause a secondary hyperpolarization of V_{mc} (decrease in IR_a drop) indicated by the transition B-C. Concomitantly, V_{ms} shifts in the mucosa-positive direction because of redistribution of transcellular and paracellular currents. Note that ΔV_{ms}(B-C) $< \Delta V_{mc}$(B-C). Thus, the control fR_a will be underestimated because fR_a(A-C) $< fR_a$(A-B). However, fR_a(A-C) is a correct estimate of fR_a at the V_{mc} value established by the clamp. Transitions C-D and C-E represent the voltage responses to brief superimposed pulses of smaller magnitude than the clamp current, applied in the *m*-to-*s* and *s*-to-*m* directions, respectively, while R_a is held constant at its pre-pulse value at C. The V_{mc}-hyperpolarizing transition C-D causes a voltage-dependent increase in R_a (transient D-F). Conversely, the depolarizing transition C-E causes an additional decrease in R_a which then elicits the transient E-G. In summary, if the voltage-dependent change in fR_a occurs within the measurement period itself, fR_a(C-G) $< fR_a$(C-E) and fR_a(C-F) $< fR_a$(C-D). Therefore, regardless of the polarity of the superimposed pulse, the true (pre-pulse) value of fR_a will be underestimated.

The transepithelial resistance fell during *m*-to-*s* current clamps. The values estimated from the superimposed pulses were close to those predicted assuming a linear change in R_t. However, during *s*-to-*m* clamps the estimates of R_t from superimposed pulses were higher than those calculated from linear interpolation between the measurements obtained at the onset and end of the clamp (Fig.6). This effect was accentuated by pulse currents in the same direction as the clamp, and when the pulses were applied during exposure to TEA^+ (Fig. 9). The changes in R_t during and after the clamp were asymmetric: R_t rose slowly during the clamp, fell initially at a rapid rate upon termination of the clamp, and continued to decrease more slowly over the next few minutes at a rate similar to that of $V_{ms}^{E_t}$ (*see* Fig. 9). These results suggest that, once elevated, R_s becomes voltage dependent. Further, when an *s*-to-*m* clamp is applied during exposure to TEA^+ the fact that R_z^{app} is unchanged at a time when R_t is markedly elevated indicates that the change in resistance is occurring at or near the tight junctions, and not along the length of the lateral intercellular spaces. Other investigators have reported similar increases in R_t in response to transepithelial voltage or current clamps (Frömter, 1972; Bindslev et al., 1974;

Reuss & Finn, 1977) and have explained this phenomenon by current-induced narrowing of the lateral spaces and/or increase in the resistivity of the fluid. Although this interpretation could be correct for the region immediately below the tight junctions, the increase in resistance of the spaces cannot be distributed along their entire length, in that, fR_a should decrease and R_z^{app} should increase (Clausen, Lewis & Diamond, 1979; Boulpaep & Sackin, 1980; Essig, 1982). Contrary to this expectation, no change in either fR_a or R_z^{app} was observed in the present studies.

Nature of the Apical Membrane Potassium Conductance

Recent patch-clamp studies have established the existence of voltage- and Ca^{2+}-activated K^+ channels in the apical membranes of a variety of epithelial cells (Hunter et al., 1986; Frindt & Palmer, 1987; Bolívar & Cereijido, 1987; Guggino et al., 1987). In *Necturus* gallbladder, indirect experimental evidence suggests that the potassium conductance of the apical membrane is increased by elevation of aCa_i. Bello-Reuss et al. (1981) demonstrated that exposure of the tissue to cyanide or the Ca^{2+} ionophore A23187, agents thought to increase aCa_i, causes an increase in apical membrane potassium conductance. In voltage-clamp experiments, García-Díaz et al. (1983) found that mucosal addition of A23187 caused a shift in the voltage-dependency of fR_a to more negative values of V_{mc} with no change in the sigmoidal relationship, indicating an increased voltage sensitivity of the apical K^+ channels. Thus, these macroscopic results suggest that a Ca^{2+}- and voltage-activated K^+ channel is present in the apical membrane in *Necturus* gallbladder. In addition, using patch-clamp techniques, a Ca^{2+}- and voltage-activated K^+ channel has been found in the apical membrane of *Triturus* gallbladder (Maruyama, Matsunaga & Hoshi, 1986). Preliminary results in *Necturus* gallbladder (Segal & Reuss, 1987) suggest the existence of apical membrane K^+ channels with a finite Na^+ permeability, which appear to be open most of the time at the baseline apical membrane voltage.

It is unclear at present whether the K^+-conductive properties of the apical membrane of *Necturus* gallbladder can be explained by a single K^+ channel. An observation suggestive of the existence of two apical K^+ channels is the difference in effects of TEA^+ and Ba^{2+}: whereas TEA^+ is a more potent blocker of the voltage sensitivity of fR_a, Ba^{2+} has a larger depolarizing effect. By itself, the lack of a significant change in membrane voltage with TEA^+ suggests the existence of a second apical K^+ channel which is TEA^+ insensitive and voltage independent. This channel may be the one responsible for the spontaneous apical membrane voltage. The presence of two apical membrane K^+ channels in *Necturus* gallbladder epithelium was proposed from current fluctuation analysis (Gögelein & Van Driessche, 1981). In addition, the results of Frindt and Palmer (1987) suggest the existence of two types of K^+ channels in the apical membrane of rat cortical collecting tubule.

Alternatively, our data could be explained on the basis of a single apical K^+ channel with a finite open probability under control conditions, and peculiar apparent affinities for the two blockers. For instance, the TEA^+ sensitivity could be greater in the depolarized region, and the Ba^{2+} effect dominate near the resting membrane voltage. Additional patch-clamp experiments and/or reconstitution studies will be necessary to resolve this issue.

In summary, we conclude that a voltage-dependent K^+ conductance is present at the apical membrane of the epithelial cells of *Necturus* gallbladder. The voltage sensitivity of this channel is a more serious problem in fR_a measurements than putative current-dependent changes in resistance of the lateral intercellular spaces. This apical K^+ channel is blocked by TEA^+, although this agent has minimal effects on the baseline electrical parameters of the epithelium. Our results also demonstrate that TEA^+ can be used as a tool to identify changes in fR_a arising from the voltage dependence of R_a.

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