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ELECTRICAL CHARACTERISTICS OF THE APICAL AND BASAL-LATERAL MEMBRANES IN THE TURTLE BLADDER EPITHELIAL CELL LAYER

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(1) The active transport of Na^+ across the turtle bladder epithelial cell layer consists of a passive entry step through a Na^+-selective path in the apical membrane and an active extrusion step through Na^+ pump-containing path in the basal-lateral membrane together with some back-leakage through the paracellular spaces and tight junctions between the epithelial cells. This hypothesis has now been verified qualitatively and to some extent, quantitatively by the use of an intracellularly-located microelectrode in conjunction with a conventional assembly of extracellularly-located macroelectrodes mainly in short-circuited bladders bathed by Na^+-rich Ringer media. Under these conditions, the intracellular potential (V_{sc}) averaged 38.4 mV with the cell electronegative; the fractional resistance of the apical membrane (fR_a) averaged 0.55; while the concomitant transepithelial parameters, short circuiting current (I_{sc}) and electrical conductance (G_t), average 68.6 $\mu A/cm^2$ and 0.98 mS/cm^2, respectively. (2) The relation between these parameters and the transepithelial flow of Na^+ (or I_{sc}) is evoked by blocking Na^+ entry into the cell (by the mucosal addition of amiloride or removal of mucosal Na^+). Amiloride-induced blockade of the Na^+ entry step results in a rapid hyperpolarization of the cell interior during which $V_{sc} = -79.1$ mV and $fR_a = 0.92$. I_{sc} and G_t (equivalent to the shunt conductance under these conditions) averaged 5 $\mu A/cm^2$ and 0.35 mS/cm^2, respectively. The entire process is reversible on re-admission of Na^+ entry into the cell. (3) A slow depolarization of the cell interior in the period of blocked transapical Na^+ entry is opposite to that expected from an electroneutral Na^+-K^+ exchanging pump; but instead is the predictable response of an electrogenic Na^+ pump in parallel with a passive K^+-selective conductance in the basal-lateral membrane. (4) The electrogenicity concept is substantiated after pretreatment of the bladder with serosal ouabain, which changes the response of V_{sc} to amiloride (from the aforementioned biphasic response) to a step-function response, attributable mainly to the development of a slowly dissipating K^+ diffusion potential across the basal-lateral membrane. (5) Under open-circuit conditions, the electronegativity of cell to mucosa (V_a) is a linear inverse function of the electropositivity of serosa to mucosa (V_t). For $V_t \geqslant 100$ mV, V_a is positive; and for V_t between −30 and 90 mV, V_a is negative.

Introduction

Background. Isolated turtle bladders possess active transport mechanisms for the reabsorption of Na^+, Cl^-, and HCO_3^- and/or for the secretion of H^+. Under open circuit conditions in Na^+-rich, (Cl^- + HCO_3^-)-containing media, the serosa is electropositive (60 to 160 mV) to the mucosa while NaCl and $NaHCO_3$ are reabsorbed as ion pairs against transepithelial gradients of chemical potential. When choline is substituted for Na^+ in such media, the serosa becomes electronegative (by 10 to 90 mV) to the mucosa while choline chloride and choline bicarbonate are also reabsorbed against chemical

potential gradients [1,2]. Under short-circuiting conditions in the absence of all transepithelial gradients of electrochemical potential, the short-circuiting current (I_{sc}) is carried by the net transepithelial flows of Na^+, Cl^-, HCO_3^- (and/or H^+). Thus I_{sc} (predominantly that of net positive charge flowing from mucosa to serosa) approximates the algebraically summated rates of Na^+, Cl^-, and HCO_3^- reabsorption (or proton secretion) in a ($Na^+ + Cl^- + HCO_3^-$)-containing bathing system. The mutual independence of these active ion transports as well as their algebraic additivity in short-circuited bladders has been demonstrated in two kinds of experiments. In the first kind, the rate of Na^+ reabsorption remains constant while I_{sc} increases after SO_4^{2-} is substituted for Cl^- and HCO_3^- in the mucosal fluid [3]; and in the second kind, the rate of Cl^- reabsorption remains constant and I_{sc} (from m to s) becomes negative after choline is substituted for Na^+ in both bathing fluids [4]. In what follows, we consider theoretical and experimental aspects of only the Na^+ transport-paths in the turtle bladder membranes.

Models. The active transepithelial flow of Na^+ can be simulated (in a first approximation) by an electrical model in which the parameters (e.m.f.s and conductances) of the apical and basal-lateral membrane are lumped into those of an electrically-equivalent, single, imaginary membrane (Fig. 1A). However, this lumped parameter system has no requirements for stimulating the mucosal sidedness of the actions of amiloride [5]; or the serosal sidedness of the actions of ouabain [6] or disulfonic stilbenes [7–9].

In order to account for these as well as for other phenomena in open-circuited and short-circuited bladders, it is necessary to expand the single membrane (or lumped parameter) model into an electrically-equivalent, two-membrane (or distributed-parameter) model (Fig. 1B). In this model, each actively transported ion flows through a corresponding ion-specific conductive path in the apical membrane, across yet another ion-specific path in the basal-lateral membrane, and across non-selective, conductive paths in the cell fluid and in the paracellular channels.

Purpose of this study. The presently reported experiments, primarily directed toward an analysis of the ion-selective paths envisaged in the two membrane model, were designed to determine how one set of these parameters, the Na^+-selective e.m.f.s and conductances, are distributed between each plasma membrane and how such a distribution can be related to the rate of the Na^+ flow across the cellular and paracellular paths of the epithelial wall. In order to evaluate such parameters experimentally it was necessary to use an intracellularly-located microelectrode in conjunction with the conventional extracellularly-located macroelectrodes under open-circuit and short-circuiting conditions.

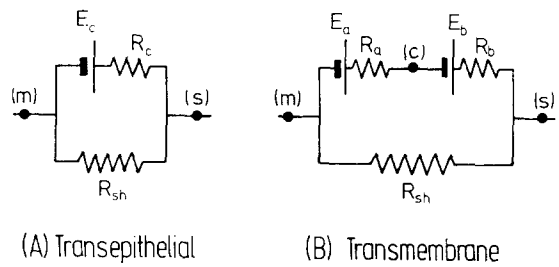

Fig. 1. Equivalent electrical analogues of the Na^+-selective (and non-selective) paths in turtle bladder epithelium. (A) The lumped parameter or imaginary single membrane model in which E_c^{Na} and G_c^{Na} denote Na^+-selective transcellular e.m.f. and conductance elements while G_{sh} denotes the non-selective paracellular or shunt conductance. (B) The distributed or two membrane model in which the transcellular parameters are subdivided into apical (a) and basal-lateral (b) components while G_{sc} is the same as above.

Hirschhorn and Frazier [10] reported the first data on transmembrane electrical parameters measured by microelectrode impalements of turtle bladder epithelia in Na^+-containing media. Aside from other limitations (see Discussion), this report did not deal with the dependency of the microelectrode parameters on the rate of Na^+ transport. In contrast, the experiments to be described in this report deal explicitly with the effects of induced changes in the rate of Na^+ reabsorption on the transmembrane and transepithelial potentials and conductances in open-circuited and short-circuited turtle bladder epithelia.

Methods

Excision and isolation of the urinary bladder of *Pseudemys scripta* turtles have been described in detail previously [4]. Microelectrode techniques,

described in detail for frog skin [11,12] were adapted for the present use in turtle bladders. These bladders (with the mucosal surface up) were first placed on the top of a metallic grid (covering the closed serosal half-chamber), then stretched gently until the visible surface foldings appeared to flatten out. The stretched state was maintained by pinning the outer boundary of the bladder to a Sylgard-filled concentric concavity in the wall of the serosal half chamber. The lower surface of the upper half chamber was coated with silicone grease and placed a few mm above the upper surface of the lower chamber. Finally, the lower chamber was slowly raised with a screw drive until that portion of the bladder surface peripheral to the metallic grid made contact with the silicone grease. Thus the bladder and grid (0.4 cm^2 in area) separated the mucosal (or upper chamber) from the serosal chamber.

Using gravity flow systems, both surfaces of the bladder were continuously and separately perfused with Ringer solution of the following composition (in mM); Na^+, 100; K^+, 5.3; Ca^{2+}, 2.0; Mg^{2+}, 0.8; Cl^-, 25; HCO_3^-, 20; SO_4^{2-}, 27.5; ($HPO_4^{2-} + H_2PO_4^-$), 0.74; glucose, 11; sucrose, 24; final pH, 7.4 after bubbling with 98% O_2 + 2% CO_2. While keeping the mucosal half chamber open to allow for microelectrode impalements of the cell from above, a negative hydrostatic pressure of 20–30 cm H_2O was applied to the serosal (or lower) side to secure the attachment of the bladder to the supporting grid. This negative pressure induced no detectable effects on the electrical parameters.

Microelectrodes for intracellular recording were prepared from Omega dot® microfiber tubing (No. 30-32-1; Frederick Haer and Co., Ann Harbor, MI). They were pulled on a mechanical, two-stage microelectrode puller (W. Klotz, Department of Physiology, Munich). Filled with 3 M KCl from behind, the input resistance of these electrodes was 20–60 MΩ and the tip potentials, generally below 5 mV. The microelectrodes were first advanced close to but not in contact with the bladder surface under the control of a dissection microscope (Leitz, F.R.G.). Then, transapical membrane impalements were made by advancing the microelectrodes (perpendicularly to the mucosal surface) with a stepping motor micromanipulator (Nano-stepper, Fa. Marcinowski, Heidelberg, F.R.G.). The sole criterion for impalement of an epithelial cell was the monitored electrical parameters.

Mechanical perturbations of the micropuncture system, which was assembled in a Faraday cage, were minimized by the following steps: a constant transmural hydrostatic pressure difference decreasing from the mucosal to the serosal surface was maintained in both chambers; and the chamber and micromanipulator were rigidly mounted to a solid baseplate on a heavy table with rubber shock absorbers. Switches, valves and liquid reservoirs (which had to be manipulated during and after the microelectrode impalements) were independently mounted.

Except when otherwise indicated, the bladders were kept short circuited using an automatic voltage clamping device similar to that of Helman and Fisher [13]. Microelectrode potentials with reference to the mucosal solution were measured with Ag/AgCl-electrodes connected to the input stage of a BB 7621 Instrumentation amplifier (input resistance > 10^{12} Ω). These signals were displayed on a Tektronic storage oscilloscope for immediate inspection and on an Esterline Angus potentiometric strip chart recorder for subsequent inspection and analysis. Input resistance of the microelectrode was measured from the voltage deflection produced by calibrated current pulses (0.3 nA, 200 ms) injected in the microelectrode. Transepithelial potential and short circuit current were monitored continuously on the Esterline Angus strip chart recorder. Values of electrical parameters (defined below) were read from digital panel meters using appropriate storage techniques, e.g. for obtaining the transient (600 ms) current responses to an imposed step-increment of transepithelial voltage.

The symbols for and operational definitions of the various electrical parameters are the following:

I_{sc}, transepithelial short-circuit current;

V_t, transepithelial potential difference (serosa with respect to mucosa);

G_t or $1/R_t$, transepithelial conductance, measured from transient (600 ms) transepithelial current response to an externally imposed +10 mV step change in V_t;

G_c and G_{sh}, conductance of transcellular and paracellular pathway, respectively;

V_a, transapical membrane potential difference (cellular with respect to mucosal fluid);

V_b, transbasal-lateral membrane potential difference (cellular with respect to serosal fluid);

fR_a, resistance of the apical membrane (R_a) divided by transcellular resistance ($R_a + R_b$), i.e. $fR_a = R_a/(R_a + R_b)$. This fraction was calculated from the measured changes in the transapical membrane potential (ΔV_a) divided by the induced, calibrated change (+10 mV) in transepithelial potential (ΔV_t), i.e. $fR_a = \Delta V_a/\Delta V_t$.

Theoretical considerations

Experimentally-induced changes in the active transport of sodium across the isolated turtle bladder epithelium can be replicated by an equivalent electrical circuit (Fig. 1A), consisting of a sodium-specific e.m.f.-containing transcellular limb (c) in parallel with a paracellular limb (sh). Because such changes in Na^+ transport occur without concomitant changes in Cl^- or HCO_3^- transport in short-circuited bladders [3,4,6], the anion transport parameters are implicitly lumped with paracellular parameters in the equivalent circuit models used in the following analysis.

From the classical laws of electrical circuit networks, it can be said that under short circuiting conditions,

$$I_{sc} = G_c \cdot E_c \tag{1a}$$

where

$$G_c = G_t - G_{sh} \tag{1b}$$

and E_c denotes the transcellular electromotive force for Na^+ transport. For a more detailed representation, the components of the transcellular limb (E_c and G_c) are divided in apical (a) and basal-lateral (b) membrane components in series while that of the paracellular limb (G_{sh}) remains the same (Fig. 1B). Since the two circuits in Fig. 1 are electrically equivalent (as defined by Thevenin's theorem), it is required that

$$E_c = E_a + E_b \tag{2a}$$

$$G_c = \frac{G_a \cdot G_b}{G_a + G_b} \quad \text{or} \quad R_c = R_a + R_b \tag{2b}$$

As used in the present analysis, changes in the rate of active Na^+ transport under short circuiting conditions (ΔI_{sc}^{Na}) are defined as those which occur with little or no change in the concomitant rates of active Cl^- and HCO_3^- (or H^+) transport. This means that for any finite I_{sc}^{Na} of the type specified, $\Delta I_{sc}^{Cl} = \Delta I_{sc}^{HCO_3} = \Delta I_{sc}^{H} = 0$, even though the absolute level of each ion flow (I_{sc}^{Cl}, $I_{sc}^{HCO_3}$ and/or I_{sc}^{H}) does make a finite contribution to the measured absolute levels of the transepithelial and transcellular electrical parameters (I_{sc}, G_t, V_{sc} and fR_a). This specified kind of change in Na^+ transport has been demonstrated experimentally; the rates of Cl^-, HCO_3^-, and/or H^+ transport do remain constant during reductions of I_{sc}^{Na} by removal of extracellular Na^+ or by the serosal addition of ouabain [3,4,6]. The stimulatory effect of amiloride on the HCO_3^- transport moiety of I_{sc} [14] will be ignored because its magnitude is much less than that of the amiloride-induced decrease in the Na^+ transport-related moiety of I_{sc} [5]. If then an Na^+-selective change in the measured short-circuiting current (I_{sc}) were a straight line function of an accompanying change in conductance (G_t), and if the simultaneously measured change in the transmembrane potential (V_{sc}) were a straight line function of the change in fractional resistance of the apical membrane (fR_a), these relations could be used to estimate: the Na^+-selective driving forces across both membranes ($E_b^{Na} + E_b^{Na}$); the resistance across the basal-lateral membrane (R_b^{Na}); as well as the remaining electrical parameters in the model of Fig. 1B. In this case, the Na^+ transport-related short-circuiting current (I_{sc}^{Na}) is defined by the following equations.

$$I_{sc}^{Na} = (E_a^{Na} + E_b^{Na})/(R_a^{Na} + R_b^{Na}) \tag{3a}$$

or

$$I_{sc}^{Na} = E_{Na}/R_{Na} = E_c/R_c \tag{3b}$$

Eqn. 3b can be substituted into Eqn. 1b to yield

$$G_t = G_{sh} + (I_{sc}^{Na}/E_c) \tag{4a}$$

as previously derived by Yonath and Civan [15].

After amiloride or removal of mucosal Na^+, the Na^+ transport-related parameters (I_{sc}^{Na} and G_t) decrease without changes in anion-related transport, which can be expressed in the form,

$$\Delta G_t = \frac{1}{E_{Na}} \cdot \Delta I_{sc}^{Na} \quad (4b)$$

when G_{sh} and E_{Na} are invariant. Therefore, $1/E_{Na}$ and G_{sh} can be estimated, respectively, from the slope of a plot of measured values of G_t versus those of I_{sc} and from the estimated conductance at the zero intercept of this plot (where $I_{sc}^{Na} = 0$).

The electromotive force (E_b^{Na}) in and the resistance (R_b^{Na}) of the basal-lateral membrane (Fig. 1B) can be estimated from data obtained with micro- and macroelectrodes under short-circuiting conditions. The potential between the intracellular compartment and the serosal compartment (V_{sc} or V_b) is defined by the equation,

$$-V_{sc} (= -V_b) = E_b^{Na} - R_b^{Na} \cdot I_{sc}^{Na} \quad (5a)$$

where R_b^{Na} is determined from the slope of a plot of values of V_{sc} versus I_{sc}. During an amiloride-induced change in I_{sc}^{Na}, it can be said that,

$$|\Delta V_{sc}/\Delta I_{sc}| = R_b^{Na} \quad (5b)$$

and that $V_{sc} = -E_b^{Na}$ *, when $I_{sc}^{Na} = 0$. The required linearity between V_{sc} and I_{sc} would be assured if the basal-lateral parameters for Cl^-, HCO_3^- and/or H^+ transport, as well as those for Na^+ transport (E_b^{Na} and R_b^{Na}) remain invariant during the course of such a change. Such a linear relationship, previously demonstrated in the frog skin [16], will be assumed to hold true in the turtle bladder.

Substituting Eqn. 3a into 5a eliminates I_{sc} and leads to the equation,

$$-V_{sc} = E_b^{Na} - (E_a^{Na} + E_b^{Na}) \cdot (1 - fR_a) \quad (6a)$$

in which fR_a is the fractional transcellular resistance of the Na^+-selective path in the apical membrane, as operationally defined. With this equation, E_{Na} can be determined from the slope of the plot of values of V_{sc} and fR_a under conditions where the resistance of the apical membrane is selectively altered. Thus,

$$|\Delta V_{sc}/\Delta fR_a| = (E_a^{Na} + E_b^{Na}) = E_{Na} \quad (6b)$$

The intercepts evaluated at $fR_a = 0$ and $fR_a = 1.0$ gives values for E_b^{Na} and E_a^{Na}, respectively.

Results

Continuously recorded values of transmembrane and transepithelial parameters were obtained before and after the mucosal addition or removal of amiloride in short-circuited and in open-circuited bladders bathed on both surfaces by identical Na^+-rich Ringer solutions unless otherwise indicated.

A. Short-circuiting conditions

In a typical experiment (Fig. 2), the initial advancement of the microelectrode into the bladder tissue (at $t < 1$ min) caused an artifactual tip potential of approx. 5 mV (electrode positive to mucosal fluid) before any transmembrane resistance barrier

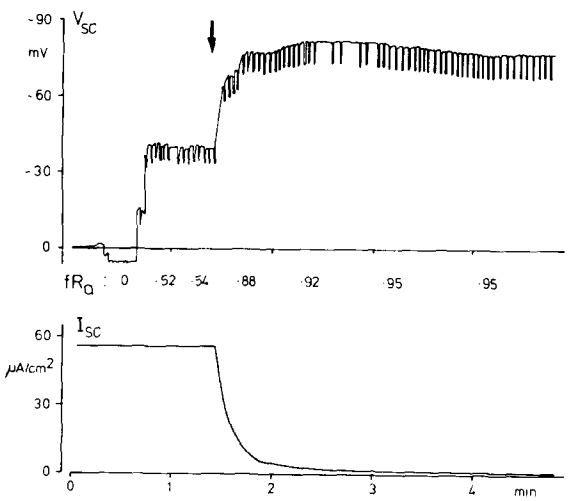

Fig. 2. Effect of mucosal addition of 10^{-5} M amiloride (arrow) on transmembranal potential (V_{sc}) and fractional apical resistance (fR_a) in upper panel; and on concomitant transepithelial short-circuiting current (I_{sc}) in lower panel. Deflection in the record of V_{sc} result from transepithelial voltage clamping to +10 mV (serosa positive).

* Note that E_b^{Na} is defined as the effective e.m.f. in the basal-lateral membrane regardless of the ionic mechanisms that contribute to its generation (K^+-diffusion potential and electrogenic Na^+ pump; vide infra).

was passed. Such a barrier was penetrated with further advancement of the microelectrode, after which the fractional resistance of the apical membrane (fR_a) and the transapical membrane potential (V_{sc}) increased abruptly to steady, sustained levels of 0.52–0.54 and –42 mV, respectively. The maintenance of these levels with a stationary microelectrode was presumptive evidence for the intracellular location of that microelectrode. Such a location was further substantiated by the subsequent electrical response to the mucosal addition of amiloride. Within 2–3 s after such an addition, V_{sc} and fR_a increased rapidly and in 2–3 min, reached final levels of –82 mV and 0.95, respectively, while I_{sc} decreased from 54 $\mu A/cm^2$ to near-zero and G_t decreased from 0.94 to 0.33 mS/cm^2. Similar effects were elicited by amiloride in 13 experiments and by removal of mucosal Na^+ (choline replacement) in three experiments. No difference was detectable between the results obtained by these two methods to reduce transapical Na^+ entry.

On the basis of such results, summarized in Table I, we were able to obtain quantitative estimates of (i) transepithelial and (ii) transmembranal parameters.

(i) Transepithelial. The mean transcellular driving force for sodium ($E_{Na} = E_a^{Na} + E_b^{Na}$), estimated from the measured amiloride-induced changes in transepithelial conductance relative to those in short circuiting current ($\Delta G_t/\Delta I_{sc}$), amounted to 100 mV (Eqn. 4b); and the mean paracellular conductance (G_{sh}), estimated from the zero intercept ($I_{sc}^{Na} = 0$) of the plot of G_t versus I_{sc} (Eqn. 4a) amounted to 0.35 mS/cm^2 (Table I). The magnitude and linearity of the slope ($\Delta G_t/\Delta I_{sc}$) were established by data from five independent experiments on the effect of mucosal amiloride. In these experiments, G_t proved to be a straight line function of I_{sc}.

TABLE I

Mean values for transepithelial parameters (G_t, I_{sc}) and transmembrane parameters (V_{sc} and fR_a) during treatment with amiloride or removal of Na^+ from the mucosal fluid.

Condition	G_t (mS/cm^2)	I_{sc} ($\mu A/cm^2$)	V_{sc}	fR_a
Control	0.90 ± 0.08	68.8 ± 7.0	–38.4 ± 3.1	0.55 ± 0.03
Amiloride	0.35 ± 0.05	5.5 ± 1.8	–79.1 ± 2.0	0.91 ± 0.01

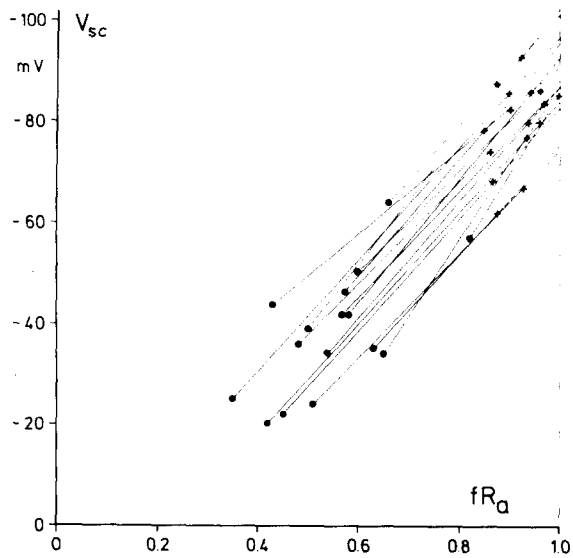

Fig. 3. V_{sc} versus fR_a in 16 short-circuited bladders. Variations in each parameter from control values (•) are those induced by 10^{-5} M amiloride or Na^+-free mucosal incubation (+). Observations from individual impalements are connected by lines.

(ii) Transmembranal. The dependency of V_{sc} on fR_a was shown to be direct in all experiments (Fig. 3). The mean value of the Na^+-selective driving force ($E_a^{Na} + E_b^{Na}$), as estimated by Eqn. 6b from measured changes in the transmembranal potential relative to those in the fractional resistance ($\Delta V_{sc}/\Delta fR_a$), amounted to 115 ± 5 mV, which is 15% greater than the value of E_{Na} estimated with macroelectrodes alone from the slope, $\Delta G_t/\Delta I_{sc}$. The mean value of E_a^{Na}, evaluated at the extrapolated locus, $fR_a = 0$, amounted to 26 ± 5 mV; and that of E_b^{Na}, evaluated at $fR_a = 1.0$, amounted to 89 ± 4 mV. The Na^+-selective resistance of the basal-lateral membrane (R_b^{Na}), estimated by Eqn. 5b from the measured changes in the transmembrane potential relative to those in the short-circuiting current ($\Delta V_{sc}/\Delta I_{sc}$), amounted to 0.72 ± 0.11 $k\Omega \cdot cm^2$ and the mean value of E_b^{Na}, evaluated at $I_{sc} = 0$ amounted to 80 ± 3 mV (see Eqn. 5a).

Alternatively, the value of the Na^+-selective resistance (R_b^{Na}) can be estimated from the product, $R_c \cdot (1 - fR_a)$, where R_c and fR_a are simultaneously determined during a single microelectrode impale-

ment, and where $(1 - fR_a) = fR_b$, by definition. In this case, the mean R_b^{Na} amounted to 0.76 ± 0.11 kΩ · cm² while the value of R_a^{Na} was 0.91 ± 0.09 kΩ · cm². The near equality of the mean R_b^{Na} estimated from the product of R_c and fR_b with that estimated from the slope $\Delta V_{sc}/\Delta I_{sc}$ indicates that: (i) the characteristics of a single cell (dependent solely on fR_a and V_{sc}) approximated those of all cells in the epithelial layer (dependent on I_{sc} and R_t), and consequently that (ii) the microelectrode voltages were not significantly shunted by leaks at the site of the impalements.

Such analyses could not be carried out completely for each and every apparently successful impalement. This was because the intracellular potential (e.g. V_a or V_{sc}) and fR_a often failed to remain constant long enough. Nevertheless, the V_{sc} and fR_a often did remain stable for more than 30 s, reaching mean values (±S.E.) of −38 ± 2 mV and 0.57 ± 0.02, respectively, in 69 impalements. It is pertinent to note that such values were similar to those in Table I; hence could be used for purposes other than analyzing all of the equivalent circuit parameters.

B. Open circuit conditions

The potential profile of the turtle bladder upon open circuit conditions was recorded (42 impalements, 10 bladders) under control conditions (n = 21) and in the presence of mucosal amiloride or removal of Na^+ from the mucosal perfusion fluid (n = 21). It was found (Fig. 4) that the transapical membrane potential (V_a) was a nearly straight-line function of the transepithelial potential (V_t); that the slope, $V_a/V_t \approx -1$; and that $V_t \approx V_b \approx 70$–$90$ mV when $V_a = 0$. This linear dependence requires that V_b remain essentially constant over the entire range of V_t, from −50 to +130 mV. For maximal values of V_t (>100 mV), the orientation of V_a was reversed by 10 to 20 mV (cell being positive to mucosa), and consequently the transepithelial potential was that of a two-step function of increasing electropositivity. For less than maximal values of V_t (<70–90 mV), the transepithelial potential profile (from mucosal to serosal fluid) was that of a trough with a negatively directed step across the apical membrane and a positively directed step across the basal-lateral membrane.

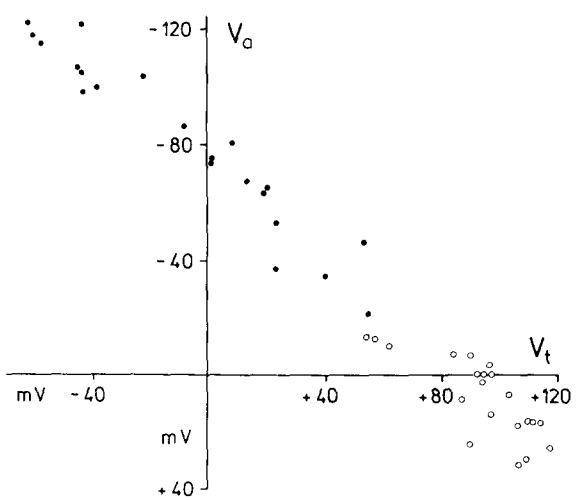

Fig. 4. V_a versus V_t in ten open-circuited bladders. Values under control conditions and those induced by 10^{-5} M amiloride or removal of mucosal Na^+ are indicated by open and filled circles, respectively.

C. Electrogenic properties of the basal-lateral membrane

The next experiments were designed to test the validity of a tentative hypothesis in which it is assumed that the transbasal-lateral membrane potential is generated by an electrogenic pump element in parallel with K^+-selective and anion-selective conductive paths. In the first period of these experiments on short-circuited bladders (n = 4) the mucosal source of Na^+ for the basal-lateral Na^+ pump was blocked by removing all exogenous Na^+ from and/or adding amiloride to the the mucosal medium. In a typical experiment (Fig. 5), the I_{sc} reached near-vanishing levels, V_{sc} reached a steady-state level of −78 mV, and fR_a reached a value of 0.94 after this manouver. Then Na^+ Ringer without amiloride was substituted for the (choline + amiloride)-containing mucosal fluid, and within 25 s, V_{sc} had depolarized from −78 mV to approx. −40 mV while fR_a had decreased from 0.94 to 0.54. In ensuing 2 min, V_{sc} repolarized to reach a final level of −53 mV while fR_a increased from 0.54 to a steady-state value of 0.62. At this point (t = 3.8 min), the Na^+-selective path in the apical membrane was again blocked by removal of Na^+ from and addition of amiloride to the mucosal fluid. V_{sc} hyperpolarized rapidly and transiently to −95 mV (a level clearly more than the previous steady state level under the

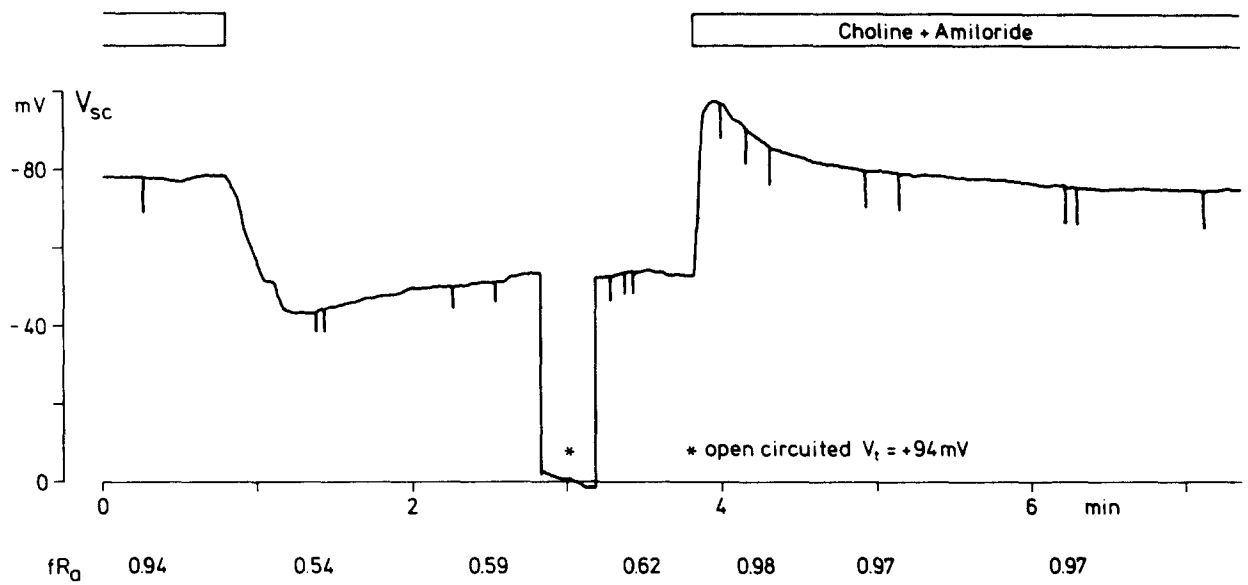

Fig. 5. Effects on V_{sc} and fR_a of (i) amiloride and removal of mucosal Na^+ (t = 0 to 1 min); (ii) addition of Na^+ to and removal of amiloride from mucosal fluid (t = 1 to 4 min); and (iii) removal of Na^+ from and addition of amiloride to the mucosal fluid (t = 4–8 min). Amiloride: 10^{-5} M.

same bathing conditions), then depolarized slowly to a new steady level of approx. −80 mV (or essentially the same as that of the previous steady state). Concomitantly (starting at t = 3.8 min), fR_a increased rapidly to 0.98 during the initial hyperpolarization and remained constant for the remaining period of slow depolarization (Fig. 5).

A tentative explanation of these data is the following. The rapidly-developed, amiloride-induced hyperpolarization of the basal-lateral membrane (Fig. 5) is presumably caused by a primary decrease in the Na^+ conductance across the apical membrane superimposed upon the electromotive force of the basal-lateral Na^+ pump element and/or that of the transbasal-lateral K^+ diffusion potential (generated by the Na^+-pump element), all of which can increase the electronegativity of the cell without changing the electromotive force of the Na^+-pump element (E_b^{Na}). The subsequent slowly developing depolarization of this membrane, which occurs concomitantly with a progressive decrease in the concentration of intracellular Na^+, leads to a progressive decrease in the Na^+ pumping rate. Finally a quasi-steady state is reached in which the magnitude of V_{sc} across the basal-lateral membrane can be attributed in part to the continued active Na transport from cell to serosal fluid. This process maintains low levels of intracellular Na^+ and high levels of intracellular K^+; and might contribute in part toward the maintenance of low intracellular levels of Cl^- and HCO_3^-.

One cannot invoke any electroneutral mechanism such as Na^+-K^+ exchange to account for the observed sequence of rapid hyperpolarization and slow depolarization of the basal-lateral membrane after the amiloride-induced block of entry of Na^+ from the mucosal into the cell fluid. This is because lowering of the cellular Na^+ concentration by the continued operation of an electroneutral exchange of Na^+ for K^+ across the basal-lateral membrane should increase the cell K^+, and consequently increase V_{sc}; but V_{sc} was decreased in these experiments. On the other hand, rapid hyperpolarization followed by slow depolarization of the basal-lateral membrane is a predictable consequence of reducing the delivery of Na^+ to an electrogenic Na^+-pump element in that membrane.

If this explanation were correct, pre-treatment of the bladder with ouabain should abolish the transient hyperpolarization in response to an amiloride-induced decrease of the apical membrane conductance. In fact, the serosal addition of ouabain

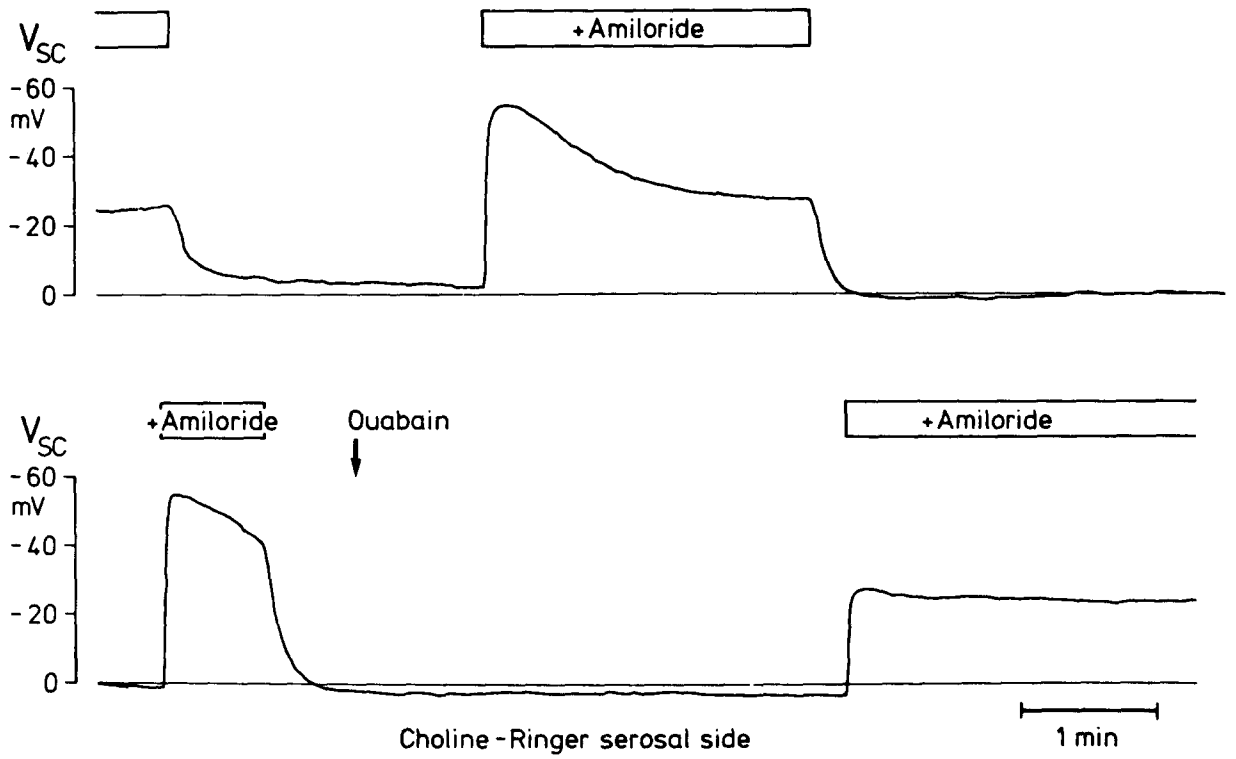

Fig. 6. Effects of V_{sc} of: mucosal addition and removal of 10^{-5} M amiloride in the absence of ouabain (upper panel and left side of lower panel); and the corresponding amiloride effect in the same bladder after a 3-min exposure to ouabain. The lower level of V_{sc} in this bladder during control states is typical for bladders incubated with Na^+-free (choline)-Ringer solution on the serosal side and compares to values of V_{sc} obtained from frog skins under similar conditions (Nagel, W., unpublished observations).

did in three experiments eliminate a previously and repeatedly elicited amiloride-induced hyperpolarization or transient increase in V_{sc} (Fig. 6). The steady state level of V_{sc} in this ouabain-treated, amiloride-blocked bladder was not different from that seen during the earlier period when amiloride had been added in the absence of ouabain.

Discussion

The intracellular potential profiles in the turtle bladder epithelia of the present experiments were similar in several ways to those obtained elsewhere from frog skin [11–13] and *Necturus* urinary bladder [17]; but not at all similar to those reported elsewhere on other turtle bladder epithelia from the same species of turtles [10]. In this connection, Hirschhorn and Frazier have reported that the transepithelial potentials in turtle bladders under open-circuited conditions were 24.8 to 57.6 mV (serosa positive) in winter and summer months, respectively, while the concomitant intracellular potentials, also electropositive with respect to the mucosal fluid, varied from 16 to 38 mV. Under short circuiting conditions in the same bladders, the intracellular potentials, reported as electronegative to the extracellular fluids, varied from –7 to –14 mV [10]. None of the values reported by Hirschhorn and Frazier are in any way comparable to those found in the present experiments (see Fig. 4 for open-circuit conditions; Table I and Fig. 3, for short-circuiting conditions). The fundamental nature of the discrepancy becomes apparent when one compares the two sets of intracellular potentials at equal levels of transepithelial potentials, in which case, the polarity of the transapical membrane potentials in the open-circuited bladders of our study is found to be opposite to that reported by

Hirschhorn and Frazier. The apparent and probably the main reason for this discrepancy is the presence or the development of leaks around the impalement sites. The effects of such leaks, previously discussed in detail by Lindemann [18], Higgins et al. [17], and Nagel [11], have been looked for and when found, excluded from the data in the present report; but no mention of a similar search for evidence of leaky impalements can be found in the report of Hirschhorn and Frazier [10].

Apart from these technical problems, the transmembranal and transepithelial flow of Na^+ in turtle bladders of the present report, as well as that in other tight epithelia [12,13,17,19] can be replicated by the flow of electric current through a specific set of conductance elements of an equivalent circuit model (Fig. 1B) in the following manner.

(i) The entry of Na^+ into the cell across the apical membrane is a passive, electrically-conductive process, the rate of which depends upon: the membrane conductance (G_a^{Na}); the transmembrane gradient of Na^+ concentration; and the transmembrane electric field (V_a).

(ii) The extrusion of Na^+ from the cell across the basal-lateral membrane is an electrically-conductive but active process, driven by a pump mechanism within that membrane. This pump, represented as a Na^+-specific electromotive force (E_b^{Na}) in series with Na^+-selective conductance paths in each plasma membrane (G_b^{Na} and G_a^{Na}), operates in a rheogenic (electrogenic) mode to render the cell interior electronegative to the serosal fluid, to lower the concentration of intracellular Na^+, and to increase that of intracellular K^+.

(iii) One consequence of this transmembrane distribution of K^+ concentration is the generation of a K^+ diffusion potential, which apparently accounts for most of the observed transbasal-lateral membrane potential *.

* In the present experiments, the magnitude of the transbasal-lateral electric potential difference (approx. 85 mV) is less than that found in frog skin [11,12,16] or *Necturus* urinary bladder [17], which can be explained by the fact that the extracellular K^+ concentration was 5.4 mM in the present experiments and 2.5 to 3.0 mM in the other experiments.

Blockade of the Na^+ entry

This model can also be made to replicate the sequence of observed electrical changes associated with a reversible turning-off of the Na^+ reabsorptive process across short-circuited turtle bladders, such as that caused by the mucosal addition of amiloride or by the removal of mucosal Na^+.

(i) The first event after amiloride addition is a rapidly developing increase in the intracellular electronegativity, which reaches a maximal or hyperpolarized level. The magnitude of this potential spike is due in part to the amiloride-induced increase in the Na^+-selective resistance of the apical membrane ($+\Delta R_a^{Na}$) and in part to the continued operation of the Na^+ pump in the basal-lateral membrane.

(ii) The next event is a slowly developing decrease (depolarization) in the magnitude of the intracellular electronegativity which reaches a level still greater than that which prevailed before amiloride. This slow and partial depolarization is due to a decreased pumping rate occasioned by a concomitant decrease in the intracellular Na concentration. Eventually the rate of Na^+ leaking (from serosa to cell) reaches that of Na^+ pumping (from cell to serosa), at which point the net rate of Na^+ reabsorption is near-zero (i.e., $I_b^{Na} = I_{sc} \approx 0$).

Electrogenicity of the Na pump

The electrogenic nature of the Na^+ pump function, established by the effect of amiloride both before and after ouabain treatments (Figs. 5 and 6), also suggests the following.

(i) Ouabain turns off the Na^+ pumping within seconds after its diffusion from the bulk serosal fluid to the Na^+-pump sites on the external surface of the basal-lateral membrane. This suggestion is consistent with the speed of onset of the ouabain-induced inhibition of the $(Na^+ + K^+)$-ATPase activity in isolated turtle bladder membranes [5], as well as with that of the ouabain-induced elimination of the relatively slow transient changes in V_{sc} after amiloride in the intact short-circuited bladders of this report (Fig. 6). A comparably rapid inhibition of Na^+ pumping in intact epithelia has also been invoked by Nagel to account for ouabain-induced changes in the electrical properties of frog skin [20] and by Zeuthen and Wright [21] to account for those in the frog choroid plexus.

(ii) The finite, persistent I_{sc} in the first 3–5 min after ouabain, which has presumably stopped all Na^+ pumping, is carried across the apical membrane by passive Na^+ flow from mucosal to cell fluid and across the basal lateral membrane by passive K^+ flow from the cell to serosal fluid, i.e. all of the I_{sc} consists of dissipative current flows occasioned by the transmembrane ion gradients and the differences in the Na^+ and K^+ permeabilities of the apical and basal lateral membrane.

(iii) However, the finite I_{sc} which persists after ouabain, vanishes within seconds after the mucosal addition of amiloride while the magnitude of the V_{sc} increases in the manner of a step function to render the cell interior more electronegative. The maintained level of this V_{sc} can be ascribed mainly to a slowly dissipating cell-to-serosal diffusion potential of K^+.

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References

1 Brodsky, W.A. and Schilb, T.P. (1965) Am. J. Physiol. 208, 46–57
2 Brodsky, W.A. and Schilb, T.P. (1966) Am. J. Physiol. 210, 987–995
3 Gonzalez, C.F., Shamoo, Y.E., Wyssbrod, H.R., Solinger, R.E. and Brodsky, W.A. (1967) Am. J. Physiol. 213, 333–340
4 Gonzalez, C.F., Shamoo, Y.E. and Brodsky, W.A. (1967) Am. J. Physiol. 212, 641–650
5 Wilczewski, T. and Brodsky, W.A. (1975) Am. J. Physiol. 228, 781–790
6 Solinger, R.E., Gonzalez, C.F., Shamoo, Y.E., Wyssbrod, H.R. and Brodsky, W.A. (1968) Am. J. Physiol. 215, 249–261
7 Ehrenspeck, G. and Brodsky, W.A. (1975) Biochim. Biophys. Acta 419, 555–558
8 Brodsky, W.A., Durham, J.H. and Ehrenspeck, G. (1979) J. Physiol. 287, 559–573
9 Cohen, L.H., Mueller, A. and Steinmetz, P.R. (1978) J. Clin. Invest. 61, 981–986
10 Hirschhorn, N. and Frazier, H.S. (1971) Am. J. Physiol. 220, 1158–1161
11 Nagel, W. (1976) Pflügers Arch. 365, 135–143
12 Nagel, W. (1977) J. Physiol. 269, 777–796
13 Helman, S.I. and Fisher, R.S. (1977) J. Gen. Physiol. 69, 571–604
14 Ehrenspeck, G., Durham, J.H. and Brodsky, W.A. (1978) Biochim. Biophys. Acta 509, 390–394
15 Yonath, F. and Civan, M.M. (1971) J. Membrane Biol. 5, 366–385
16 Nagel, W. (1978) J. Membrane Biol. 42, 99–122
17 Higgins, J.T., Jr., Gebler, B. and Frömter, E. (1977) Pflügers Arch. 371, 87–91
18 Lindemann, B. (1975) Biophys. J. 15, 1161–1164
19 Lewis, S.A., Wills, K.K. and Eaton, D.D. (1978) J. Membrane Biol. 41, 117–148
20 Nagel, W. (1980) Biochim. Biophys. Acta 599, 736–740
21 Zeuthen, T. and Wright, E.M. (1978) Biochim. Biophys. Acta 511, 517–522