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Relationship of Transient Electrical Properties to Active Sodium Transport by Toad Urinary Bladder

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Summary. Application of voltage pulses of 10 mV for periods of 9 sec across toad urinary bladder elicits a rapid deflection in transepithelial current. Frequently, the current decays back towards its baseline value during the course of the polarizing pulse. This transient phenomenon can be induced, or its magnitude increased, by raising the mucosal or serosal Na^+ concentration. The transient can be abolished by sufficiently hyperpolarizing the tissue (rendering serosa positive to mucosa), by inhibiting transcellular Na^+ transport with amiloride or ouabain, and by increasing the serosal K^+ concentration. Vasopressin increases net Na^+ movement across toad bladder but does not elicit these transients. It is proposed as a working hypothesis for further study that the transient behavior characterized in this study reflects: (1) the partition of Na^+ between the apical plasma membrane and contiguous fluid layers, (2) the partition of K^+ between the basolateral plasma membrane and adjacent submucosal fluid layer, and (3) the negative feedback interaction between intracellular Na^+ activity and Na^+ permeability of the apical plasma membrane of the transporting cells.

Isolated epithelia have long served as useful models of transepithelial transport (e.g., Matteucci & Cima, 1845; Reid, 1890; Huf, 1935). Electrophysiologic studies of such epithelia have been largely concerned with the DC properties of the tissues (Koefoed-Johnsen & Ussing, 1958). This approach has indeed proved fruitful. For example, measurement of changes induced in DC resistance has helped characterize sites and modes of action of the hormones vasopressin (Civan, Kedem & Leaf, 1966) and aldosterone (Civan & Hoffman, 1971; Saito & Essig, 1973). On the other hand, a number of investigators have remarked on certain non steady-state electrical properties of epithelia which do not readily conform to the simplest possible model of a single effective capacitance and resistance in parallel (e.g., Tercafs & Schoffeniels, 1961; Janáček, 1963; Finkelstein, 1964; Ussing & Windhager, 1964; Civan, 1970; Kidder & Rehm, 1970; Bindslev, Tormey & Wright, 1974; Reuss & Finn, 1977).

The urinary bladder of the toad has constituted a particularly favorable preparation for studies of sodium transport for several reasons. Transepithelial sodium transport can be monitored electrically under many experimental conditions because of the equivalence of short-circuit current and net sodium transport (Leaf, Anderson & Page, 1958), at least in subspecies of *Bufo marinus* obtained from the Dominican Republic (Davies, Martin & Sharp, 1968). The geometry of the transporting epithelial cells is appreciably simpler than that of amphibian skin (DiBona, Civan & Leaf, 1969). In addition, the tissue responds vigorously to hormones and pharmacologic agents which affect transport across mammalian epithelia (Leaf, 1965).

Application of pulses of constant current or voltage to toad bladder can elicit complex changes in the conjugate electrical parameter. For example, in response to step pulses of constant current, lasting seconds to minutes, the transepithelial voltage may slowly rise to a maximal value, may exhibit reasonably squarewave behavior, or slowly decay from an initial peak deflection (Civan, 1970). These transient electrical properties depend upon the current density and polarity of the stimulus. However, their precise basis has been obscure, in part because of the experimental difficulty in reproducibly eliciting the phenomena. In the present manuscript, we report data further characterizing these transient properties, and suggest one possible interpretation as a working model for further study.

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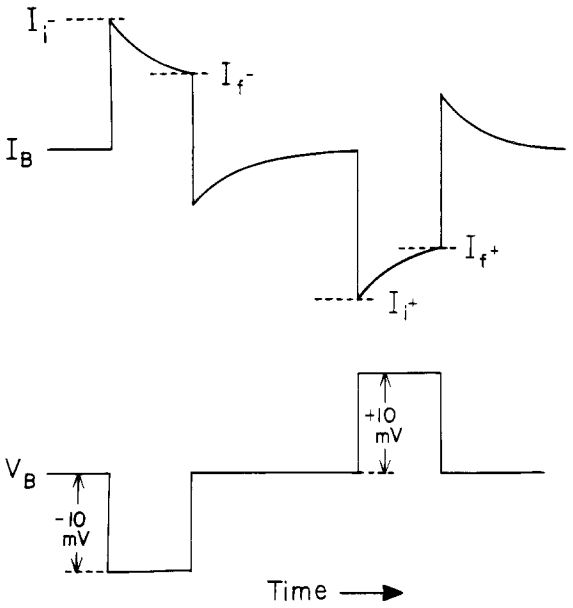

Fig. 1. Definition of parameters used in analyzing current transients following small steps in clamping voltage

Materials and Methods

Female specimens of the toad *Bufo marinus* were obtained from the Dominican Republic (National Reagents, Inc., Bridgeport, Conn.) and maintained on sphagnum moss. Paired experimental and control tissues from the same toad were provided by mounting each hemibladder between the two halves of a separate cylindrical Lucite chamber. The half-chamber making contact with the mucosal surface was fitted with an O-ring 4.4 cm in diameter. The medium-containing core of the chamber had an inner diameter of 1.3 cm; thus, 1.3 cm^2 of tissue were studied in each preparation. The intervening Lucite annulus was coated with silicone rubber grease (High Vacuum Grease, Dow Corning Corp., Midland, Mich.) to reduce edge damage (Higgins et al., 1975; Lewis & Diamond, 1976; Civan & DiBona, 1978). The serosal half-chamber was also treated with silicone rubber grease.

The serosal surface of the tissues was supported by nylon mesh. The height of the mucosal reservoir always exceeded that of the serosal medium in order to maintain a slight gradient of hydrostatic pressure ($\sim$1 cm) across the preparation.

In order to ensure uniformity of current density and to minimize polarization effects at the electrode surfaces (Schwan, 1957), current was passed between discs of platinum coated with platinum black and inserted at each end of the chamber. Voltage was sensed with miniature calomel electrodes (Fisher Scientific Company, Pittsburgh, Pa.) mounted directly in the walls of the chamber close to the plane of the tissue. The electrodes were filled with Ringer's solution, rather than saturated KCl solution, to avoid possible effects arising from prolonged diffusion of KCl into the bathing media. The presence of steady-state diffusion potentials, associated with asymmetrical bathing media, did not interfere with analysis of the electrical transients. As reported in the *Results*, transient behavior could be promptly abolished by suitable inhibitors, even in the presence of solution asymmetries. Furthermore, separate measurements of the asymmetry potentials arising from the various changes of solution indicated that the voltage differences generated were never greater than 5 mV.

Solutions and Chemicals

The standard isotonic sodium Ringer's solution consisted of (mM): Na^+, 115,1; K^+, 3.3; Ca^{2+}, 0.8; Cl^-, 113.9; HCO_3^-, 2.2; HPO_4^{2-}, 1.8; $H_2PO_4^-$, 0.3; the pH was 7.7–8.1 and tonicity 218–226 mosmol/kg water. Isotonic choline Ringer's and potassium Ringer's solutions had compositions similar to that of the sodium Ringer's solution, except for the equimolar replacement of Na^+ by choline ions and K^+, respectively. Isotonic Ringer's solutions of variable Na^+ concentration were conveniently prepared by mixing appropriate proportions of sodium Ringer's and either choline or potassium Ringer's solutions.

The hypotonic sodium Ringer's solution used in the present study consisted of (mM): Na^+, 12.0; K^+, 3.9; Ca^{2+}, 0.8; Cl^-, 11.2; HCO_3^-, 2.4; HPO_4^{2-}, 1.8; $H_2PO_4^-$, 0.3; the pH was 7.6–7.7 and the osmotic strength was 27–28 mosmol/kg water. A hypotonic choline-sodium Ringer's solution of similar composition was also used, in which 10 mM choline substituted for 10 mM Na^+.

In chloride substitution experiments, tissues were bathed with isotonic and hypotonic isethionate Ringer's solutions. The isotonic solution was similar to the standard sodium Ringer's solution except for the equimolar replacement of chloride with isethionate ions. The hypotonic isethionate solution consisted of (mM): Na^+, 14.4; K^+, 0.4; Ca^{2+}, 0.07; isethionate, 10.3; HCO_3^-, 0.2; HPO_4^{2-}, 2.0; $H_2PO_4^-$, 0.3; Cl^-, 0.1; the pH was 7.8 and tonicity 26–27 mosmol/kg water.

Vasopressin was obtained in the form of "*P*-grade" lysine vasopressin (Calbiochem, San Diego, Calif.), and amiloride was generously provided by Dr. George M. Fanelli, Jr. (Merck Institute for Therapeutic Research, West Point, Pa).

Electrical Measurements

The transepithelial potential (V, serosa positive with respect to mucosa) was initially clamped at a reference potential V_B, except for periods of 9 sec every 30 sec during which V_B was alternately increased and decreased by 10 mV. Generally, the reference potential was zero. However, during many experiments, V_B was intermittently changed to a different value for 3–10 min of measurement before being restored to zero. The transepithelial currents through both experimental and control tissues were constantly monitored on a dual-pen paper chart recorder.

Results

Current Transients Following Small Brief Voltage Steps

As the baseline value of the voltage clamp was periodically increased (or decreased) by 10 mV from the reference potential of zero, corresponding decreases (or increases) in transepithelial current were observed (Fig. 1). However, the waveform behavior of the current pulses frequently deviated from the squarewave pattern of the imposed voltage signals. Characteristically, the current rapidly underwent a maximal change from its baseline value (I_B) to a value I_i and then decayed back towards the baseline with a half time of seconds to minutes to the value I_f observed at the conclusion of the voltage pulse (Fig. 1). The results to be presented are primarily qualitative in

Table 1. Changes induced in the magnitude of the transient to brief small voltage steps

Protocol	Hemibladders A (periods)					Hemibladders B (periods)				
	1	2	3	4	5	1	2	3	4	5
Ouabain (7)	0.25 ±0.087	0.49 ±0.12	0.51 +0.14	0.16 ±0.095		0.18 ±0.068	0.37 ±0.10	0.39 ±0.11	0.45 ±0.13	
Hypotonic mucosal solution (8)	0.10 ±0.031	0.54 ±0.12				0.07 ±0.032	0.07 ±0.033			
Serosal Na^+ (5)	−0.17 ±0.010	0.36 ±0.092	0.34 ±0.13	0.00 ±0.00		−0.14 ±0.024	0.22 ±0.046	0.24 ±0.041	0.23 ±0.054	
Isethionate (3)	0.15 ±0.063	0.24 ±0.046				0.23 ±0.081	0.22 ±0.060			
Mucosal K^+ (4)	0.12 ±0.044	0.040 ±0.026	0.070 ±0.031	0.41 ±0.16		0.15 ±0.084	0.083 ±0.039	0.068 ±0.030	0.16 ±0.061	
Serosal K^+ (4)	0.32 ±0.14	−0.045 ±0.018	−0.12 ±0.03	0.31 ±0.16	0.33 ±0.17	0.24 ±0.12	0.24 ±0.08	0.44 ±0.04	0.13 ±0.07	0.21 ±0.08
Amiloride (6)	0.27 ±0.075	0.56 ±0.12	0.00 ±0.00			0.43 ±0.14	0.65 ±0.14	0.61 ±0.16		
Vasopressin (7)	0.30 ±0.084	0.14 ±0.042	0.03 ±0.011	0.03 ±0.011	0.04 ±0.011	0.26 ±0.11	0.24 ±0.11	0.22 ±0.11	0.21 ±0.11	0.45 ±0.11

The values tabulated are the means ±SE of the transient fraction parameter. The number in parentheses indicate the number of experiments performed with a given protocol. The symbols *A* and *B* simply refer to the two paired hemibladders of each experiment. The periods 1–5 refer to consecutive periods of study, which were not necessarily of equal duration. *Ouabain periods 1–4* refer to measurements: *(1)* under baseline conditions, *(2)* after addition of mucosal Na^+ to both *A* and *B*, *(3)* just before adding ouabain to *A*, and *(4)* at the conclusion of the ouabain effect. *Hypotonic periods 1–2* refer to measurements: *(1)* before and *(2)* after adding mucosal Na^+ to *A* but not to *B*. *Serosal Na^+ periods 1–4* refer to measurements: *(1)* before and *(2)* after adding serosal Na^+ to *A* and *B*, and *(3)* before and *(4)* after adding amiloride to hemibladders *A*. *Isethionate periods 1–2* refer to measurements in isethionate Ringer's solution: *(1)* before and *(2)* after adding mucosal Na^+ to *A* and *B*. *Mucosal K^+ periods 1–4* refer to measurements: *(1)* under baseline conditions, *(2)* after adding mucosal K^+ to *A*, *(3)* under baseline conditions once again, and *(4)* after adding mucosal Na^+ to *A*. *Amiloride periods 1–3* refer to measurements: *(1)* under baseline conditions, *(2)* after adding mucosal Na^+ to *A*, and *(3)* after adding mucosal Na^+ and amiloride to *A*. *Vasopressin periods 1–5* refer to measurements: *(1)* under baseline conditions, *(2)* at the peak natriferic response after adding vasopressin to *A*, and *(3)* at the conclusion of the vasopressin effect on current, and *(4)* just before and *(5)* after adding mucosal Na^+ to *B*. *Serosal K^+ periods 1–5* refer to measurements: *(1)* before and *(2)* after increasing the serosal K^+ concentration of *A*, *(3)* after then adding Na^+ to *A* and *B*, *(4)* under baseline conditions again and *(5)* after adding mucosal Na^+ to *A* and *B*. Further details concerning each protocol are summarized in the text and figure legends.

nature. However, in order to provide a semiquantitative index, a transient fraction parameter (f_{tr}) has been introduced. For depolarizing pulses (rendering the serosal voltage more negative with respect to the mucosal voltage),

$$f_{tr} \equiv \frac{I_i^- - I_f^-}{I_i^- - I_B} \tag{1}$$

while for hyperpolarizing pulses,

$$f_{tr} \equiv \frac{I_f^+ - I_i^+}{I_b - I_i^+} \tag{2}$$

where the superscripts " − " and " + " refer to values measured during depolarizations and hyperpolarizations, respectively. The effects on f_{tr} of a number of experimental manipulations are tabulated in Table 1; unless otherwise stated, values are presented as means ±SE. A summary of the qualitative results is provided in Table 2.

A transient fraction of zero indicates that no transient was observed, while $f_{tr}=1$ would indicate that the current response to a voltage step was entirely transient. The transient electrical response was sometimes sufficiently prominent to obscure even the basic patterns of the current response to the voltage pulses at the stimulus frequency applied (lower panel, Fig. 3).

Several observations indicated that this transient behavior could not have simply reflected an artifact of the recording system. First, such transients could not be elicited across a sheet of perforated Parafilm substituted for the toad bladder. Second, it proved

Table 2. Summary of qualitative changes induced in transient

Experimental procedure	Change in transient
↑ Mucosal Na^+ concentration	↑
↑ Serosal Na^+ concentration	↑
↓ Mucosal osmolality	↔
Chloride replacement by isethionate	↔[a]
↑ Mucosal K^+ concentration	sl. ↓
Transepithelial hyperpolarization	↓
Vasopressin	↓
Ouabain	↓
Amiloride	↓
↑ Serosal K^+ Concentration	↓

[a] However, functional properties of transient are altered.

possible to systematically elicit or abolish these electrical transients. Third, very substantial spontaneous changes with time could sometimes be observed in the current waveforms from the same tissue. In fact, time-dependent spontaneous changes presented a formidable obstacle to a systematic analysis of the transient electrical properties of toad bladder. Specifically, even when tissues manifested striking transient electrical behavior at the onset of an experiment, the current waveforms would usually become increasingly square-wave with time.

Ionic Dependence of Transients

The single most effective technique for eliciting the current transient following small steps in transepithelial voltage was to abruptly increase the sodium concentration of the mucosal medium. Approximately two-thirds of the preparations examined developed clearly discernible transients after the sodium concentrations were raised from 10 mM or less to values several times higher (Figs. 2–4). For example, before addition of mucosal sodium, the upper trace of Fig. 2*A* displayed very little transient behavior, and the lower trace none at all. Following elevation of the mucosal sodium concentration from 10.5 to 62.8 mM, the transient was so large and decayed so slowly that the current did not achieve a steady-state value during the brief intervals between the voltage pulses.

The osmotic pressure and ionic strength of the mucosal medium did not seem to strongly influence the development of the current transients following small brief polarizing steps. Most of the mucosal ionic substitutions were performed while maintaining the value of osmotic pressure and ionic strength equal to those of the serosal sodium Ringer's solution. However, in 8 experiments, transients were elicited in the presence of a mucosal osmolality of 27–28 mosmol. Transients were present even when the initial mucosal media contained 2 mM Na^+ and 10 mM choline. However, their magnitudes were substantially increased when choline was replaced by sodium, raising the total sodium concentration to 12 mM while maintaining the osmolality and ionic strength constant. The average value for f_{tr} rose from 0.10 ± 0.031 to 0.54 ± 0.12 on the experimental side, and remained unchanged on the control side (Table 1).

Increasing the sodium concentration of the serosal medium can also elicit transient behavior. In a series of 5 experiments, the mucosal and serosal media of both paired hemibladders initially consisted of a mixture of sodium and choline Ringer's solution containing 10.5 mM Na^+. Raising the serosal sodium concentration to 62.8 mM increased the short-circuit current, reduced the transepithelial resistance, and elicited transient behavior (Table 1). The apparently paradoxical role of serosal sodium in enhancing mucosal-to-serosal current flow has long been appreciated (Leaf, 1965), and may reflect sodium-calcium exchange across the basolateral membranes of the transporting cells. Increasing the chemical gradient favoring Na^+ influx into the cells from the serosal medium may enhance calcium extrusion, lower the intracellular activity of Ca^{2+}, and enhance Na^+ entry from the mucosal medium into the cells (Grinstein & Erlij, 1978).

Chloride ions need not be present in the external bathing media for transient behavior to be expressed. In 4 experiments, clear transients were displayed, even following the equimolar substitution of isethionate for chloride. Averaging the observations for all 8 hemibladders, f_{tr} was 0.19 ± 0.036. However, in these experiments, increasing the mucosal Na^+ concentration from 14.4 to 64.9 mM did not increase the size of the transients; the change in f_{tr} induced by adding mucosal sodium to 6 hemibladders was 0.04 ± 0.049.

Potassium has been thought to play an important role with respect to electrophysiological transients noted with certain other preparations (Janáček, 1963; Reuss & Finn, 1977). In the present study, substantial changes in the mucosal potassium concentration were entirely ineffective in eliciting transients in toad bladder. In 4 experiments, the mucosal medium consisted of an isotonic mixture of choline and sodium Ringer's solutions containing only 2 mM sodium. Equimolar mucosal substitution of potassium for choline reduced the short-circuit current and tissue conductance, and produced no transients. However, after restoring the original choline Ringer's solution to the mucosal bath, transients could be elicited by the equimolar replacement of sodium for choline (Table 1).

High serosal concentrations of potassium have been reported to abolish certain transient characteristics of frog skin (Janáček, 1963) and *Necturus* gall-

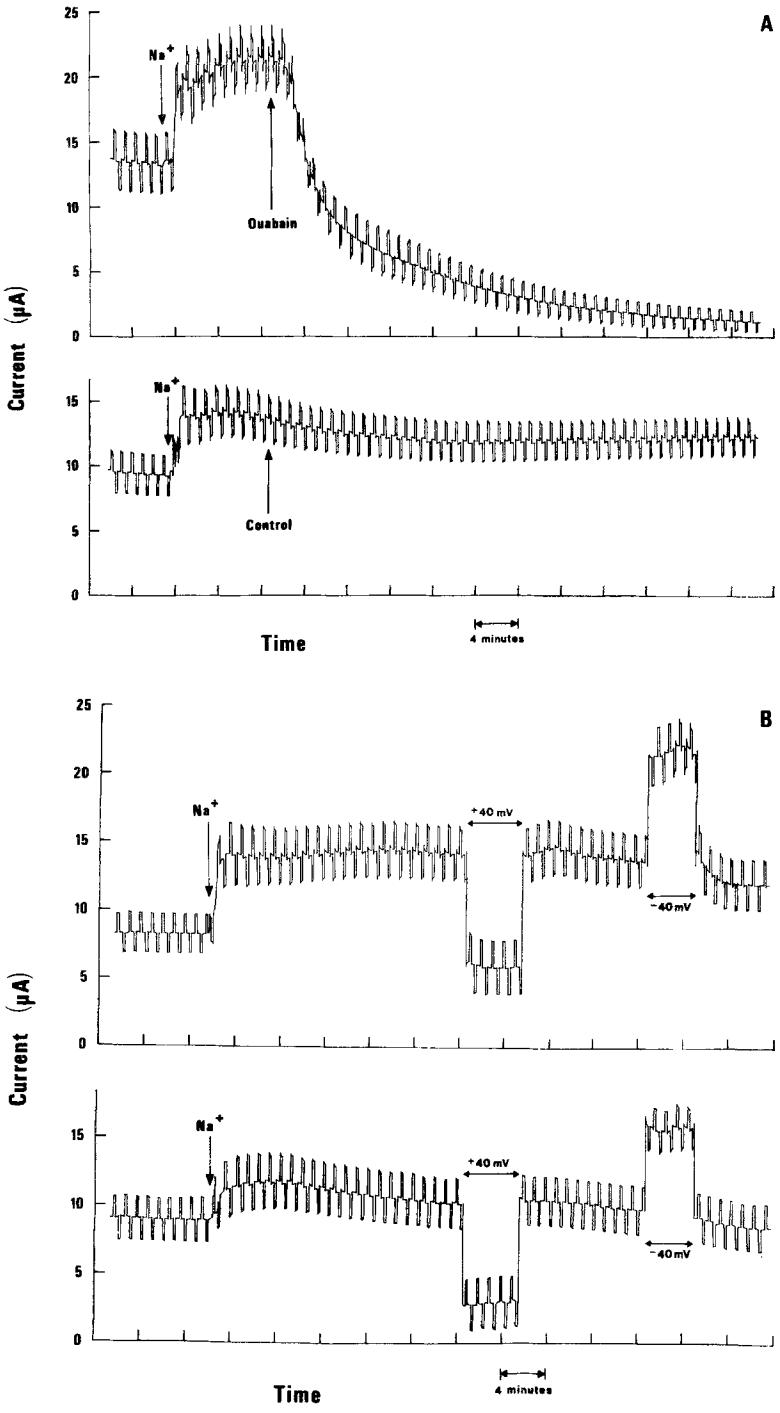

Fig. 2. Effects of mucosal sodium, ouabain, and baseline transepithelial potential on transient behavior. *(A)*: Sodium Ringer's solution bathed the serosal surfaces of both experimental and control hemibladders throughout the period of study. At the first arrows, sodium Ringer's solution was added to the initial mucosal medium, consisting of a mixture of choline and sodium Ringer's solutions, raising the sodium concentration from 10.5 to 62.8 mM. Transients to the brief small voltage pulses were produced in the control tissue (lower trace); f_{tr} rose from 0.02 to 0.23. Transient behavior was also markedly accentuated in the experimental tissue (upper trace); f_{tr} rose from 0.08 to 0.45. At the second set of arrows, ouabain dissolved in sodium Ringer's solution was added to a final serosal concentration of 4.8×10^{-4} M, eventually abolishing the transient behavior of the experimental hemibladder; addition of an identical volume of sodium Ringer's solution to the serosal medium bathing the control hemibladder was without effect. *(B)*: The media bathing the tissues of *A* were replaced with fresh solutions, and transients were elicited once again by increasing the mucosal sodium concentration. Sustained hyperpolarization to +40 mV reduced transient behavior, while depolarization to −40 mV clearly and reversibly increased the magnitude of the transients to the voltage pulses in these tissues

bladder (Reuss & Finn, 1977). However, this superficially simple manueuver depolarizes the basolateral membrane of frog skin (Fuchs, Larsen & Lindemann, 1977) and probably of toad bladder, and by reducing the serosal Na^+ concentration, likely reduces the intracellular Ca^{2+} activity, changing the state of the tissue (Grinstein & Erlij, 1978). In the presence of serosal Cl^-, the cell volume of the transporting cells increases (Robinson & Macknight, 1976*b*), and both transepithelial resistance and open circuit potential fall (Robinson & Macknight, 1976*a*). These multiple changes in the tissue induced by replacing serosal Na^+ by K^+ complicate interpretation of the experimental results.

Despite these reservations, the effect of high concentrations of serosal K^+ were examined in 4 ex-

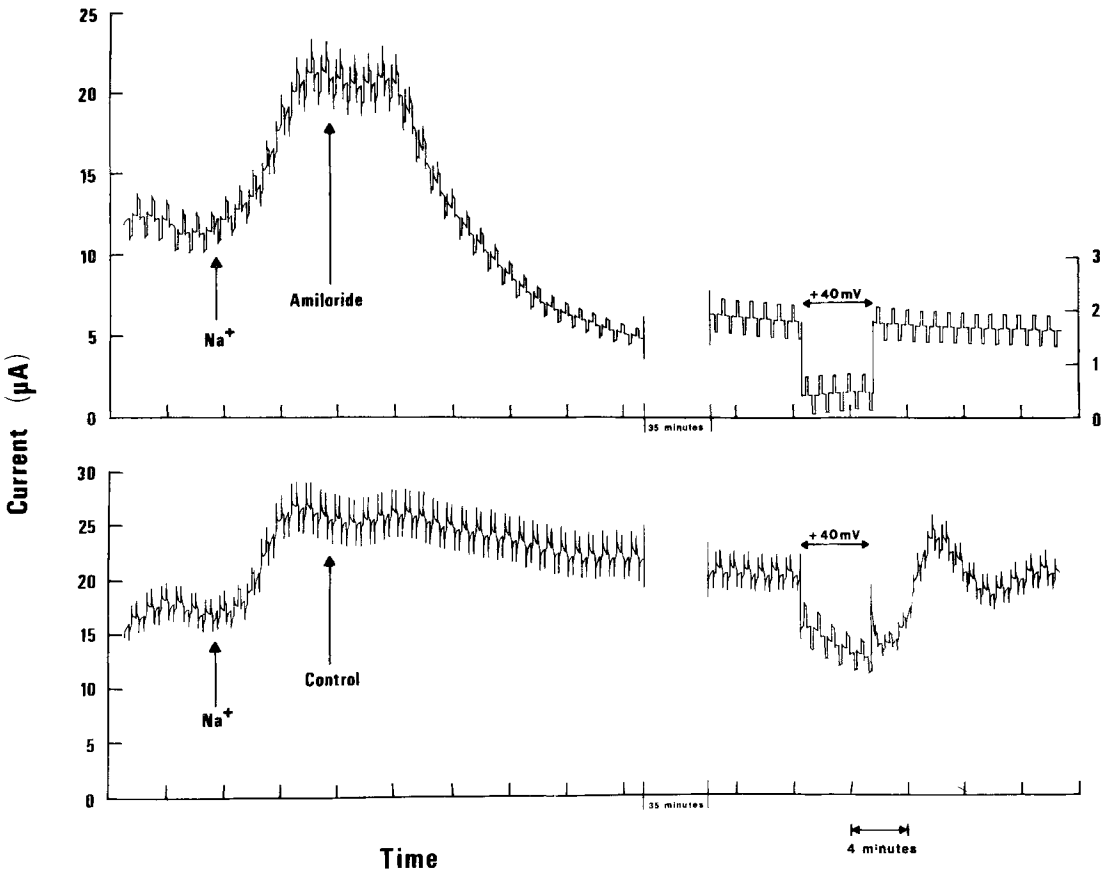

Fig. 3. Effects of amiloride and transepithelial potential on transient behavior. The hemibladders were bathed with a mixture of choline and sodium Ringer's solutions on their mucosal surfaces and the standard sodium Ringer's solution on their serosal surfaces. Isotonically increasing the mucosal sodium concentration from 10.5 to 62.8 mM increased the magnitudes of the transients. After a delay introduced by the size of the mucosal reservoir and the circulation rate of the bath, 2×10^{-5} M amiloride abolished the transient. The final portion of the figure illustrates the contrasting responses of the current traces to a sustained hyperpolarization in the presence and absence of amiloride. The terminal portion of the upper trace is presented on an expanded ordinate scale to facilitate comparison

periments (Table 1). Paired hemibladders were initially bathed with a choline-Na^+ Ringer's solution containing 10.5 mM Na^+ on their mucosal surfaces and a choline-Na^+ Ringer's solution containing 57.6 mM Na^+ and 3.3 mM K^+ on their serosal surfaces. The serosal solution of the experimental hemibladder was subsequently replaced with a K^+-Na^+ Ringer's solution, increasing the serosal K^+ concentration to 59.2 mM without altering the Na^+ concentration. The high K^+ increased the tissue conductance and abolished the baseline transient phenomenon, actually producing a small negative J_{tr}. Furthermore, when the mucosal Na^+ concentration of each hemibladder was increased from 10.5 to 62.8 mM, the transient fraction was increased on the control side, but no transient appeared on the experimental side. These effects of high serosal K^+ were reversible. When the tissues were bathed with the initial media, the experimental hemibladder once again displayed transients.

Effects of Large Steps in Clamping Voltage on Transients Following Brief Small Voltage Steps

The transients following brief small polarizing steps could be reversibly abolished by imposing sufficiently large hyperpolarizations across the preparation. For example, clamping the tissue at 40 mV reversibly abolished the transient behavior of the upper trace of Fig. 2*B*. On the other hand, transients can still be discerned in the lower trace of Fig. 3 even after application of 40 mV across the preparation. In such cases, a voltage clamp of 80 or 120 mV was necessary to elicit squarewave behavior. When transients were not present, application of positive voltage clamps of 40, 80 and 120 mV did not appear to markedly influence squarewave behavior (e.g., upper trace of Fig. 3).

The effect of negative voltage clamps, rendering the serosal medium negative with respect to the mu-

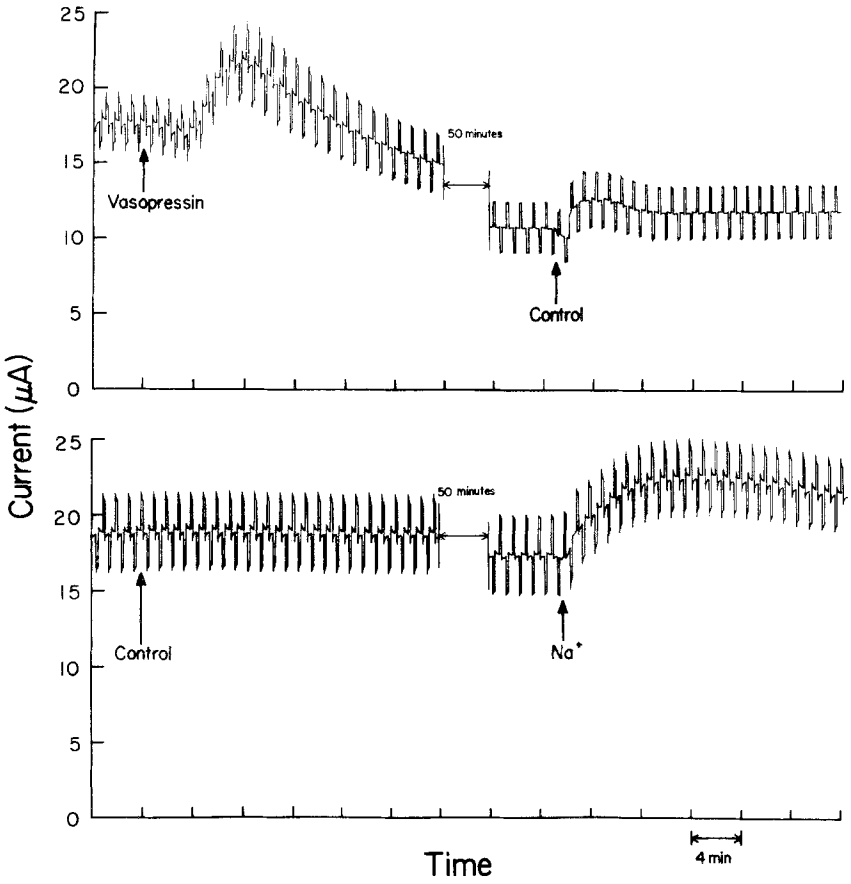

Fig. 4. Effects of vasopressin and mucosal sodium on transient behavior. The serosal media were sodium Ringer's solution. A mixture of choline and sodium Ringer's solutions containing 10.5 mM Na^+ bathed the mucosal surfaces. Addition of vasopressin to a final serosal concentration of 100 mU/ml increased the short-circuit current. However, the transient fraction fell from 0.50 to 0.30 measured at the peak current response. The transient behavior was largely gone some 23 min after adding hormone and f_{tr} was 0.00 after 60 min. Little change was noted in the control trace over the same period. In the second half of the experiment, elevation of mucosal sodium to 62.8 mM produced an increase in short-circuit current of the control hemibladder, comparable to that induced by vasopressin earlier in the experimental tissue. In contrast to the effect of vasopressin, addition of mucosal sodium increased the magnitude of the transient; f_{tr} rose from 0.10 to 0.37

cosal, was more difficult to assess. On occasion, small transients could be elicited by application of −40 mV (Fig. 2*B*). However, larger negative values appeared either to introduce complex time behavior in the current trace, obscuring the form of the waveforms, or actual irreversible tissue changes. Therefore after a series of preliminary studies, negative voltage changes were not routinely applied.

The present work has been primarily concerned with the transient electrical phenomena noted during the 9 sec following small step changes of 10 mV in voltage across toad bladder. However, substantial changes in the electrical parameters were also observed during periods of minutes following larger step changes in voltage of 40 to 120 mV (e.g., Fig. 3).

Effects of Inhibitors of Transepithelial Sodium Transport

Perhaps the most striking result obtained during the present study was the prompt and complete abolishment of transient behavior by inhibitors of transepithelial sodium transport. This observation is in agreement with reports that dinitrophenol (Tercafs & Schoffeniels, 1961) and ouabain (Janáček, 1963) can abolish a current-induced voltage transient across frog skin.

Amiloride is thought to inhibit net sodium transport specifically by blocking sodium entry from the mucosal medium into the transporting cells (Bentley, 1968; Ehrlich & Crabbé, 1968; Dörge & Nagel, 1970;

Salako & Smith, 1970*a, b;* Biber, 1971, Macknight, Civan & Leaf, 1975; Hong & Essig, 1976). In a series of 5 experiments (Fig. 3), transients were first established by increasing the mucosal sodium concentration from 10.5 to 62.8 mM. Then, 600 μl of sodium Ringer's solution containing 10^{-3} M amiloride were added to the 30 ml of mucosal medium bathing the experimental hemibladder, proving a final amiloride concentration of 2×10^{-5} M. An identical volume of sodium Ringer's solution was added to the mucosal reservoir of the control hemibladder. Amiloride consistently reduced the short-circuit current, increased the transepithelial resistance, and abolished the current transients to small brief polarizing pulses (Fig. 3).

Sodium transport can also be inhibited by ouabain, an agent thought to selectively block active extrusion from the cell into the serosal medium (Herrera, 1966). In a series of 7 experiments (Fig. 2), transients were initially established by raising the sodium concentration of the mucosal medium. Ouabain dissolved in sodium Ringer's solution was subsequently added to the serosal medium of the experimental hemibladder to a final concentration of 4.8×10^{-4} M. An identical volume of sodium Ringer's solution was added to the serosal medium of the control hemibladder. As in the case of amiloride, ouabain inhibited the short-circuit current, increased the transepithelial electrical resistance, and abolished the transients. Although not always the case after applying inhibitors, the transients could occasionally be elicited once again after removing ouabain by bathing the serosal surface several times with fresh Ringer's solution (Fig. 2).

Effect of Vasopressin

The results thus far presented might suggest that in general those maneuvers (such as increasing the mucosal and serosal Na^+ concentrations and depolarizing the tissue) which increase transepithelial sodium transport are also likely to elicit current transients. Conversely, those agents or procedures (such as amiloride, ouabain, and transepithelial hyperpolarizations) which depress net sodium transport would also be likely to abolish transient behavior. The hormone vasopressin stimulates net sodium transport across toad bladder (Leaf, 1960), probably largely by increasing Na^+ entry into the cells from the mucosal medium (Civan et al., 1966; Civan & Frazier, 1968; Civan, 1970; Macknight, Leaf & Civan, 1970; Macknight, Leaf & Civan, 1971; Yonath & Civan, 1971; Handler, Preston & Orloff, 1972). On this basis, vasopressin would be expected to elicit transient behavior. Actually, repeated efforts to confirm this expectation were unsuccessful.

This point is illustrated by the results of a series of 7 experiments (Fig. 4). The initial mucosal medium bathing each hemibladder was a mixture of choline and sodium Ringer's solution containing 10.5 mM Na^+. As was commonly the case, sodium Ringer's solution bathed the serosal surfaces. Before the addition of vasopressin, the current traces of both hemibladders of Fig. 4 exhibited transient behavior. Vasopressin, dissolved in sodium Ringer's solution, was then added to the serosal bath of the experimental hemibladder to a final concentration of 100 mU/ml; an identical volume of sodium Ringer's solution was added to the serosal medium of the control hemibladder. Vasopressin increased the short-circuit current and decreased the transepithelial resistance, as anticipated. However, the hormone also seemed to *reduce* the magnitude of the transient behavior. The transient fraction fell from an initial value of 0.50 to 0.30 by the time of peak natriferic effect, and to 0.00 some 60 min after adding hormone. Thus, the hormonal effect on transient behavior is very different from simply adding mucosal sodium. When a comparable absolute and relative increase in short-circuit current was subsequently elicited from the control tissue by raising the mucosal sodium concentration to 62.8 mM, the magnitude of the transients was clearly increased; f_{tr} rose from 0.10 to 0.37.

Discussion

Properties of Transient

The present study characterizes the transient response of transepithelial current primarily to small, brief changes in voltage across the urinary bladder of the toad. The most frequently observed form of the response was a prompt deflection, followed by a decay towards the initial baseline over a period of seconds to minutes (Fig. 1).

The transient studied seems to be specifically related to sodium transport (Table 2). It can be elicited by increasing the sodium concentration of the mucosal or serosal medium, and perhaps by sufficiently large depolarizing steps (rendering serosa negative to mucosa). The transient can be abolished by maneuvers which inhibit net transepithelial sodium movement from mucosa to serosa: (i) reducing the mucosal sodium concentration, (ii) hyperpolarizing the tissue, (iii) blocking mucosal sodium entry with amiloride, and (iv) blocking serosal extrusion of sodium with ouabain.

Transient behavior is modified, but not abolished by substituting isethionate for chloride in the bulk external media. Increasing the mucosal potassium concentration does not elicit transients. Increasing the serosal potassium concentration abolishes baseline transient behavior.

Much of the transient electrophysiological behavior of *Necturus* gallbladder has been ascribed to changes induced in the apical intercellular "tight" junctions and lateral intercellular spaces (Bindslev et al., 1974; Reuss & Finn, 1977). The permeability through the apical intercellular junctions can be minimized by reducing the osmolality of the mucosal medium with respect to that of the serosal bath. The permeability is maximized over the physiological range by making the mucosal and serosal media isosmotic (Civan & DiBona, 1978; Finn & Bright, 1978), In the present study, transients could be elicited in the presence of both hypotonic and isotonic mucosal media.

The appearance and disappearance of transient behavior are frequently, but not necessarily, associated with increase and decrease, respectively, of mucosal-to-serosal sodium transport. With time, transient behavior commonly disappears spontaneously, in the absence of clear changes in short-circuit current or transepithelial resistance. In addition, vasopressin markedly increases transepithelial sodium transport, but does not elicit transients.

Simplest Model

It is highly likely that the transient behavior summarized above reflects some from of transport number effect (Barry, 1977). Toad urinary bladder presents at least five series barriers to the transcellular passage of Na^+ from the mucosal to serosal bulk media: the mucosal and serosal unstirred fluid layers, the apical and basolateral plasma membranes of the transporting cells, and the intracellular fluid compartment. Unless the transport number for Na^+ were constant throughout the preparation, altering the transepithelial current would inevitably lead to depletion of Na^+ from one or more compartments and accumulation in one or more others (Kedem & Katchalsky, 1963).

The simplest model involving a transport number effect would be based on transient changes induced in one of the two series plasma membranes and an adjacent fluid layer. For example, a depolarizing voltage pulse would increase mucosal Na^+ entry, tending to deplete Na^+ from the mucosal unstirred layer and to accumulate Na^+ within the apical membrane. This net displacement of Na^+ could induce an interfacial polarization potential and polarization impedance (Kortüm, 1965), opposing continued Na^+ transfer, and leading to the observed transient decay in transepithelial current. A hyperpolarizing pulse would likely reduce the concentration of Na^+ in the apical membrane and increase that in the unstirred mucosal layer, tending to enhance subsequent Na^+ entry and transport with time. Such changes in the partition of Na^+ between the apical plasma membrane and contiguous fluid layers are very likely responsible, in part, for the observed transient.

Potassium may also be playing a roughly analogous role at the basolateral surface of the membrane. If extrusion of cell Na^+ into the serosal unstirred layer is coupled to K^+ accumulation by the cell (Koefoed-Johnsen & Ussing, 1958), the increased Na^+ transport produced by voltage depolarizations would lead to depletion of K^+ from the unstirred layer, opposing continued Na^+ transfer.

This simple concept does not, however, readily accommodate all the results, specifically those obtained with vasopressin. The hormone selectively increases the permeability of the apical membrane to Na^+. Therefore, we would expect vasopressin to increase the difference in transport number characterizing Na^+ in the apical membrane and adjacent layer of unstirred fluid. The increased Na^+ transport induced by the hormone would also be expected to further deplete K^+ from the unstirred fluid layer in contact with the basolateral membrane. Thus, the simplest model predicts that vasopressin should increase the magnitude of the transients, contrary to observation. Some additional mechanism must be playing a role.

Working Hypothesis

Several lines of evidence indicate that intracellular sodium reduces the sodium permeability of the apical plasma membrane of transporting epithelial cells (Morel & Leblanc, 1975; Leblanc & Morel, 1975; Cuthbert & Shum, 1976; Turnheim, Frizzell & Schultz, 1978). We suggest that the transient behavior described in the present manuscript in part reflects this negative feedback phenomenon.

Let us consider a urinary hemibladder whose transepithelial potential is clamped at 0 mV. Abruptly raising the mucosal sodium concentration four- to fivefold from an initial value of $\leqq 10$ mM will increase the rate of net sodium entry into the transporting epithelial cells. Some of this excess intracellular sodium will then be extruded across the basolateral membrane, causing an increase in short-circuit current (Frazier, Dempsey & Leaf, 1962). Not all of the excess intracellular sodium will be extruded. The residuum will remain in the cell, balanced electrically by net uptake of available anion, usually Cl^- (Macknight, 1977), and by a net extrusion of K^+ (Robinson & Macknight, 1976*c*), both largely across the basolateral membrane. A brief voltage step further depolarizing the transepithelial potential, will also render the intracellular fluid more negative to the mucosal medium, enhancing the rate of net sodium entry, and further increasing the intracellular sodium concentra-

tion. If increasing sodium concentration within the cell reduces the sodium permeability of the apical membrane, the rate of sodium entry should slow. In addition, the slight increase in intracellular sodium will reduce the electrochemical driving force across the apical membrane, further slowing sodium entry. The rate of delivery of sodium to the basolateral pump would be thereby reduced and, under certain but not all conditions, the rate of sodium extrusion would fall, producing the observed transient decay in transepithelial current. At the conclusion of the depolarizing pulse, the short-circuit current would fall below the pre-pulse baseline because of the reduced apical sodium permeability. With continued extrusion of sodium from the cell, the intracellular sodium concentration would fall, releasing the inhibition to apical sodium entry as well as increasing the electrochemical gradient favoring sodium entry, and eventually restoring the short-circuit current close to its initial value.

Hyperpolarizing pulses would induce a reverse sequence of events. Apical sodium entry would be initially reduced, decreasing the intracellular sodium concentration, increasing the apical sodium permeability, and thus increasing sodium availability to the sodium pump with time. The transepithelial current would therefore increase during the course of the hyperpolarization. At the conclusion of the hyperpolarizing pulse, the short circuit would transiently overshoot the baseline level, until the apical Na^+ permeability decayed to its initial value.

According to this model, transient behavior should depend upon: (i) the difference in electrochemical potential ($\Delta\tilde{\mu}_{Na}$) between the mucosal and intracellular fluids; (ii) the permeability to Na^+ (P^m_{Na}) of the apical plasma membrane; (iii) the functional integrity of the negative feedback system at the apical membrane; (iv) the absolute activity of intracellular Na^+ (a^c_{Na}); (v) the range of intracellular Na^+ concentrations over which this negative feedback operates, and (vi) the capacity and K_m for intracellular Na^+ of the Na^+ pump at the basolateral membrane.

We may now interpret the observed results within the framework of the working hypothesis. Changes in transepithelial potential and mucosal Na^+ concentration will alter $\Delta\tilde{\mu}_{Na}$. Changes in the serosal Na^+ concentration probably alter P^m_{Na} (Grinstein & Erlij, 1978). Both factors will affect net Na^+ entry into the cell.

Amiloride probably acts both by reducing P^m_{Na} and by interrupting the physiologic feedback relationship between a^c_{Na} and P^m_{Na}. Although vasopressin increases P^m_{Na}, it is ineffective in inducing transients probably because, like amiloride, it interferes with the $a^c_{Na} - P^m_{Na}$ feedback system. In addition, since vasopressin reduces the electrical resistance of the apical membrane relative to that of the basolateral membrane (Civan & Frazier, 1968), a smaller fraction of the total transepithelial voltage pulse will be imposed across the apical membrane.

Ouabain inhibits basolateral Na^+ extrusion, elevating a^c_{Na}, and probably saturating the apical feedback mechanism.

The spontaneous disappearance of transients very likely reflects the delicate interrelationship between the rates of apical entry and basolateral extrusion of sodium. A number of factors may play a role. For example, the absolute values of the apical membrane potential (V^m) and of P^m_{Na} may well fluctuate with time, altering the baseline rates of mucosal sodium entry. The change (δV^m) in V^m induced by the transepithelial voltage pulses of magnitude δV may also vary, because of its dependence on the relative resistances of the apical and basolateral membranes. However, the simplest interpretation would be that following addition of mucosal sodium, intracellular sodium activity increases with time. Thus continued aplication of polarizing pulses should induce smaller fractional changes in a^c_{Na}. These smaller fractional variations are likely to be less effective in eliciting the changes in P^m_{Na} which, in part, provides the presumed basis for the transients described.

The interpretation presented is not unique. However, it does accommodate the current observations and provides a working hypothesis to be tested by direct measurements of intracellular sodium activity.

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