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Norepinephrine stimulation of sodium transport in *Necturus* urinary bladder

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Norepinephrine alters the transepithelial electrical properties of an open-circuited urinary bladder from the mud puppy, *Necturus maculosus*. When 10^{-5} M norepinephrine is superfused over the serosa of the epithelium, the transepithelial voltage (V_t) and short-circuit current (I_{sc}) increase as the resistance (R_t) decreases. The norepinephrine-mediated changes are reversed by the addition of amiloride ($5 \cdot 10^{-5}$ M) to the mucosal Ringer's solution. The serosal adrenoceptors mediating the Na^+ transport are more sensitive to norepinephrine ($EC_{50} = 1.2 \cdot 10^{-6}$ M) than to epinephrine or isoproterenol. Since the I_{sc} is blocked selectively by the antagonist, phenoxybenzamine, stimulation of active transepithelial Na^+-flux by catecholamines is mediated by an α-adrenoceptor. The apical cell membrane voltage (V_a) and fractional resistance (fR_a) were recorded using conventional KCl-filled microelectrodes. Untreated tissues have V_a close to 0 mV while the basolateral membrane voltage (V_b) is between −85 and −95 mV. About 90% of R_t is apical cell membrane resistance (fR_a). When amiloride inhibits sodium transport, V_a becomes negative, V_b hyperpolarizes slightly and fR_a increases to 97%. On the other hand, if the bladders are treated with norepinephrine, fR_a decreases to 79% as V_a becomes positive and V_b depolarizes. When R_t changes, the resistance of the paracellular pathway (R_p) is unaltered. Changes in the electrical properties of the tissue appear to be mediated primarily by alterations in R_a. Since the *Necturus* bladder does not respond to antidiuretic hormone, this study implies that biogenic amines regulate Na^+ transport in the epithelium.

Introduction

Bentley and Heller [1] recorded small amiloride- and ouabain-sensitive transepithelial voltages (V_t) and short-ciruit currents (I_{sc}) in isolated urinary bladders from *Necturus maculosus*. Amphotericin-B stimulated but arginine-vasotocin (AVT) had no effect on V_t and I_{sc}. Although the salamander possesses an in vivo response to aldosterone, the steroid did not stimulate Na^+ transport in isolated bladder preparations [2]. Higgins et al. [3] found an inverse relationship between open-circuit V_t and transepithelial resistance (R_t). When 'edge-damage' is minimized in the high-resistance epithelium, the rate of Na^+-transport (indicated by I_{sc}) is directly correlated with tissue conductance (i.e., $1/R_t$). Apparently, the primary mechanism regulating bladder Na^+ transport involves altering transcellular resistance.

Microelectrode studies in the tissue [4–6] found a correlation between apical/basolateral membrane resistance and epithelial transport rate. Bladders with low V_t and high R_t have electronegative apical membrane voltages (V_a; mucosal ground). On the other hand, tissues with larger V_t and lower R_t have positive V_a. In either state, the basolateral membrane voltage (V_b) is always near 90 mV (cytoplasm negative). Amiloride increases R_a to 220 $k\Omega \cdot cm^2$, decreases I_{sc}, depolarizes V_t and hyperpolarizes V_b by about 6 mV [5,7]. Hyperpolarization of V_b is due to a decrease in the $I \cdot R$ drop across the resistance of the basolateral membrane (R_b). Paracellular resistance (R_p) was calculated to be at least 34 $k\Omega \cdot cm_2$. Although Nagel et al. [8] found amiloride to influence R_p in the frog skin, the pattern of the diuretic's action in *Necturus* bladder does not indicate any paracellular effect [5]. The polarity of V_a correlates with the fractional resistance of the apical membrane (fR_a). This 'outer' plasma membrane of the epithelium is essentially Na^+ permselective and, as such, represents a Na^+-dependent resistor, R_a [5]. The Na^+-entry step across the outer cell membrane conforms to the Gold-

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man-Hodgkin-Katz constant field equation for single ion diffusion [6,7]. Diffusion of Na^+ down its electrochemical gradient is apparently regulated by apical membrane permeability and potential difference. A high apical conductance increases the availability of Na^+ at the basolateral site for active pumping across the membrane. Low apical conductance decreases cytoplasmic Na^+ availability. Since both V_a and V_b vary in proportion to I_{sc}, increases in Na^+ transport should occur simultaneously with increases in both apical and basolateral membrane conductance [5,9].

The basolateral membrane of the epithelium is primarily K^+ permselective [10,11]. Smaller permeabilities for Cl^- and some unknown ion(s) parallel the basolateral membrane K^+ conductance. Previous estimates of a high basolateral Cl^- permeability are believed to be erroneous due to experimental artifacts [11,12]. Blocking basolateral K^+ conductance with Ba^{2+} reduces both net transepithelial Na^+ flux and apical membrane conductance. Changes in basolateral K^+ conductance are transmitted quickly to the apical membrane [5,7,11]. The electrical properties of the *Necturus* urinary bladder are dominated by the respective Na^+ and K^+ permeabilities of the apical and basolateral cell membranes of the epithelium. Changes in the conductance of one cell membrane in the epithelium are relayed to the other. This process may serve as crosstalk regulating transepithelial Na^+ transport [9,12].

Na^+-transport inhibitors have provided clues about the mechanisms regulating ion transport in the tissue. However, no substance other than amphotericin-B [2] is known to increase Na^+ transport in vitro. Unfortunately, this drug produces non-specific, amiloride-insensitive Na^+ influx [13]. We have found that catecholamines stimulate amiloride-sensitive Na^+ transport through the epithelium. Activating α-adrenoceptors on the basolateral membrane increases I_{sc} and V_t while decreasing R_t. Norepinephrine increases the conductance of both the apical and basolateral cell membranes while depolarizing V_a. Since exogenous cyclic AMP (cAMP) and theophylline fail to stimulate the I_{sc}, we suspect that adrenoceptor may be coupled to Na^+ transport by some mechanism that does not activate adenylate cyclase.

Materials and Methods

Necturus maculosus obtained from either Riverside Biological, Somerset, or Nasco, Ft. Garrison, WI were kept in a circulating aquarium at 6 °C. Only 'winter' animals purchased between October and April were used in experiments. The mud puppies were fed minnows and their water treated with 0.6 mg/l Methylene blue to suppress fungal growth. Urodeles were anesthesized by submersion in a Ringer's solution containing 0.1% tricaine methane sulfonate (Sigma Chemical Co., St. Louis, MO). Urinary bladders were filled with Ringer's solution, ligated, extirpated and mounted in a modified Ussing chamber [3]. After mounting, Ringer's solution covered a 3.14 cm^2 area of the bladder's mucosal surface. The serosal surace of the tissue was gravity-superfused with Ringer's (2–3 ml per min). All experiments were conducted at 23–25 °C.

A Ringer's solution with the following composition (in mM) was used in all experiments: Na^+ 109, K^+ 2.5, Ca^{2+} 1.0, Cl^- 110, HCO_3^- 2.5 and glucose 5. After bubbling with 100% O_2, the pH of the solutiuon was adjusted with 0.1 M HCl to range between 7.4 and 7.6. Ascorbic acid (100 mg/l) was added to solutions as a catecholamine preservative. Amiloride (a gift from C.A. Stone; Merck, Sharp & Dohme, West Point, PA) was stored as a 10^{-3} M solution in distilled water and diluted to $5 \cdot 10^{-5}$ M with Ringer's before use. (−)Epinephrine-(+)bitartrate, (±)isoproterenol-HCl, (±)norepinephrine-HCl, dl-propranolol-HCl, N^6,2′-*O*-dibutyryladenosine 3′:5′-cyclic monophosphate (cAMP), and theophylline were purchased from Sigma Chemical Co. (St. Louis, MO) and prepared daily. Phenyoxybenzamine was gift from Smith, Kline & French (Philadelphia, PA).

Open-circuit transepithelial and membrane voltages were measured as previously described by Higgins and Frömter [3–6]. To measure resistance, constant-current pulses (1-s duration) were passed to the tissue via Ag|AgCl electrodes from either a Tektronic model 160 pulse generator (Beaverton, Oregon) or a Physiologic Instruments model VCC 600 voltage clamp (Houston, TX). Microelectrodes were fashioned from fiber-containing borosilicate glass capillaries with either a David Kopf 700D (Tujuna, CA) vertical or an Industrial Science Associates, Inc. (Ridgewood, NY), horizontal puller. After backfilling with 3.0 M KCl, only electrodes with tip resistances greater than 30 Megaohm were used for impalements. To measure cellular membrane voltages, the pipettes were connected to either a Keithley 604 (Cleveland, OH) or WPI M701 (New Haven, CT) electrometer and maneuvered with a micromanipulator. All measurements were recorded on a Metrawatt-Servogor rectilinear two-channel recorder (Vienna, Austria).

Cell membrane (R_a and R_b) and paracellular (R_p) resistances were estimated using the circuit analysis techniques of Frömter and Gebler [5] and Demarest and Finn [11]. A simple equivalent electric circuit of the epithelium is shown in Fig. 1. According to this model, when a current, I, is passed from serosa to mucosa, the $I \cdot R$ drop from mucosa to serosa and from mucosa to cell can be used to obtain transepithelial resistance (R_t), fractional or relative resistance of the apical membrane (fR_a) and a voltage divider ratio (a). Assuming that

$$V_t = V_a + V_b$$

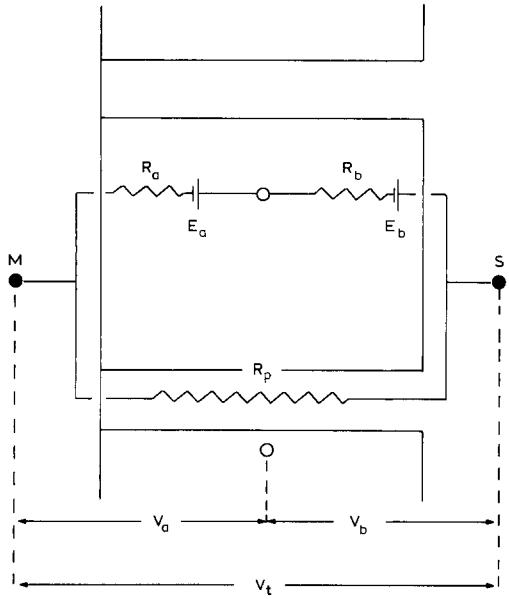

Fig. 1. Equivalent circuit analog for sodium transport in *Necturus* urinary bladder (modified from Ref. 5). R_a, R_b and R_p are apical, basolateral and paracellular resistances, respectively. E_a and E_b are electromotive forces across the apical and basolateral membrane, respectively. V_a, V_b and V_t are apical, basolateral and transepithelial voltages, respectively. M = mucosa; S = serosa. Open circle = cell.

and

$$V_b = V_t - V_a$$

The voltage divider ratio (a) is

$$a = R_a/R_b = \Delta V_a/\Delta V_b$$

where ΔV represents a change in voltage associated with current injection. In Fig. 1

$$1/R_t = (1/R_p) + (1/(R_a + R_b))$$

In this epithelium where R_p is large compared to R_a or R_b [5], fractional resistance (fR_a) is

$$fR_a = \Delta V_a/\Delta V_t$$

These parameters were used to calculate the resistance elements illustrated in Fig. 1: R_a, R_b and R_p as well as the apical membrane resistance following amiloride (R'_a) [5]. Results are reported as mean ± standard error of the mean ($\bar{X} \pm$ S.E.) unless otherwise stated. Levels of statistical significance were determined by Student's t-test. Affinity constants for dose-response curves were determined by probit analysis [14].

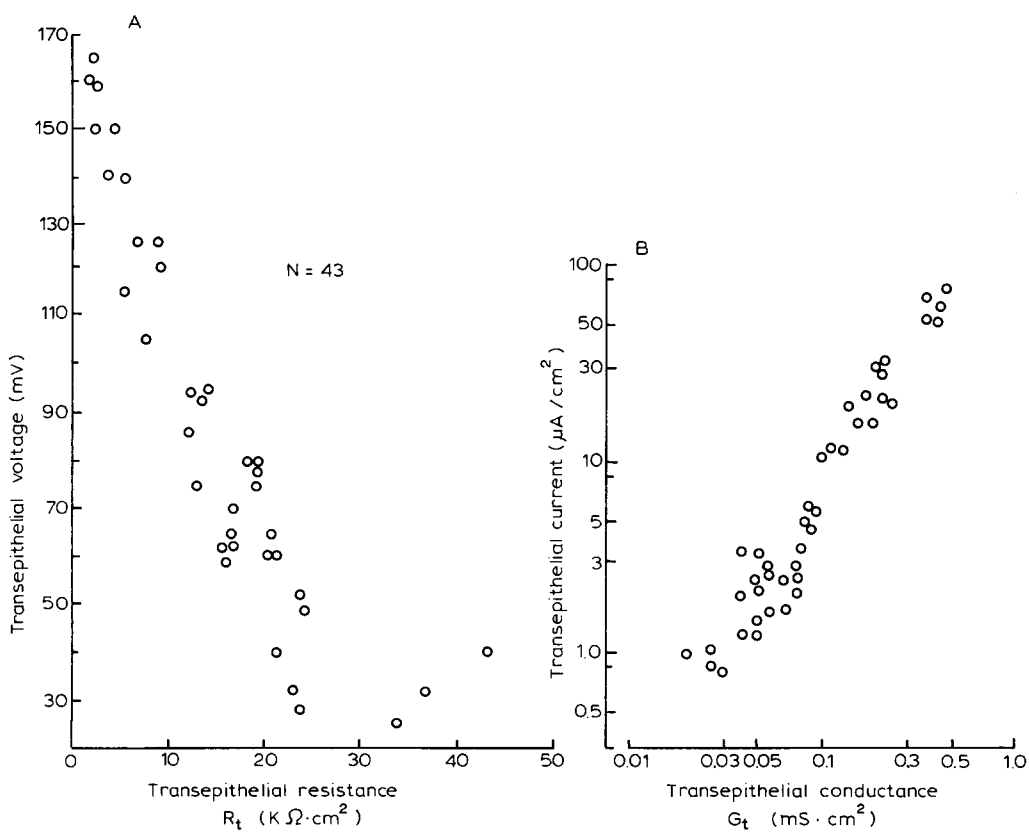

Fig. 2. (A) Relationship between spontaneous open-circuit transepithelial voltage (mV) and transepithelial resistance (kΩ·cm²) in 43 urinary bladders. (B) Relationship between transepithelial conductance ($1/R_t$) and short-circuit current in the same tissues.

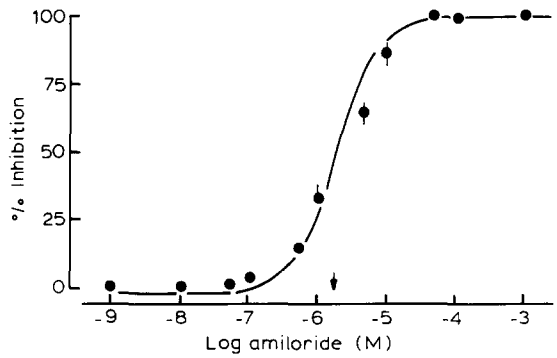

Fig. 3. Amiloride dose-response curve for inhibition of transepithelial voltage ($N = 4$). Arrow indicates median effective concentration of $1.2 \cdot 10^{-6}$ M.

Results

Transepithelial effects of catecholamines

During these experiments, only 'winter' animals were used. These usually have much higher R_t and V_t than mud puppies collected during the summer [9,15]. Fig. 2A illustrates the inverse relationship observed between transepithelial voltage and resistance. As V_t ranged from 25 to 165 mV, R_t varied between 44 and 2.2 $k\Omega \cdot cm^2$, respectively. Short-circuit current (I_{sc}) varied as a logarithmic function of transepithelial slope conductance (Fig. 2B). Amiloride in the apical Ringer's solution depolarized V_t while increasing R_t and decreasing I_{sc}. We were not able to detect amiloride effects below 10^{-7} M. Maximum inhibition of V_t is seen with $5 \cdot 10^{-5}$ M amiloride. Between these concentrations, the diuretic inhibits V_t in a dose-dependent fashion (Fig. 3). The median-effective concentration (EC_{50}) is near 1 μM. In the presence of a maximally inhibiting amiloride dose, V_t depolarized from 78 to 7 mV, R_t, increased from 16 to 40 $k\Omega \cdot cm^2$ as the I_{sc} decreased from 4.8 to 0.2 $\mu A/cm^2$ (Table I).

Norepinephrine (10^{-5} M) was added to the serosal Ringer's reservoir and perfused through the Ussing chamber. V_t and I_{sc} began to increase while R_t decreased within 5 min. These changes maximized in 30 min (Fig. 4). Apical applications of norepinephrine were without effect. Neither cAMP (10^{-3} M; $n = 3$) nor theophylline (10^{-3} M; $n = 4$) had any effect in similar experiments. In nine urinary bladders, norepinephrine increased V_t by 37 mV and I_{sc} by 9 $\mu A/cm^2$ while reducing R_t by 6 $k\Omega \cdot cm^2$ (Table I). These responses are blocked by $5 \cdot 10^{-5}$ M amiloride (see below). The stimulation of I_{sc} in the mud puppy urinary bladder is dose-dependent with a half-maximal norepinephrine concentration (EC_{50}) of $(1.2 \pm 0.9) \cdot 10^{-6}$ M (Fig. 5). Half-maximal stimulation of I_{sc} by epinephrine and isoproterenol was observed at $(8.3 \pm 0.3) \cdot 10^{-6}$ M and $(2.6 \pm 0.4) \cdot 10^{-5}$ M, respectively. The I_{sc} response to $5 \cdot 10^{-5}$ M norepinephrine is blunted by 10^{-5} M phenoxybenzamine but not 10^{-5} M propranolol (Table II). These findings imply that Na^+ transport in the *Necturus*

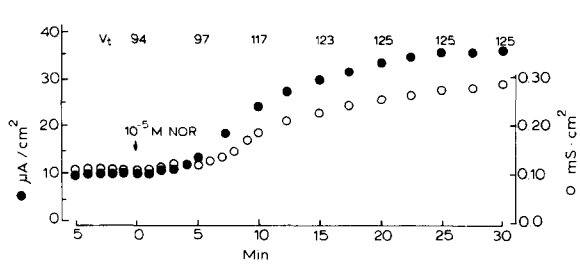

Fig. 4. Effect of 10^{-5} M norepinephrine on the transepithelial electrical properties of a *Necturus* urinary bladder. Norepinephrine (NOR) added at time = 0 min. Solid dots, short-circuit current; open dots, transepithelial conductance; V_t = transepithelial voltage.

TABLE I

Effect of amiloride ($5 \cdot 10^{-5}$ M; $N = 7$) and norepinephrine (10^{-5} M; $n = 9$) on the transepithelial electrical properties of Necturus urinary bladders

Treatment	V_t (mV)	R_t ($k\Omega \cdot cm^2$)	I_{sc} ($\mu A/cm^2$)
Control	-78.6 ± 9.4	16.1 ± 2.4	4.8 ± 0.9
Amiloride	-6.9 ± 1.2	40.1 ± 2.5	0.2 ± 0.3
Effect	-72.1 ± 1.0	$+24.2 \pm 4.5$	-4.3 ± 0.8
Control	-81.9 ± 10.3	13.6 ± 1.4	6.1 ± 0.5
Norepinephrine	-118.5 ± 4.0	7.5 ± 0.6	15.2 ± 0.4
Effect	$+37.0 \pm 7.5$	-5.9 ± 0.1	$+9.1 \pm 0.6$

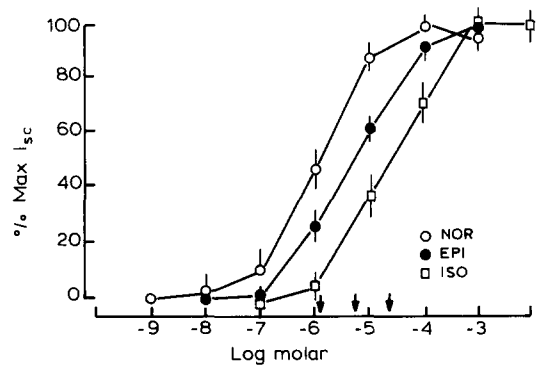

Fig. 5. Dose-response effects of norepinephrine (○; $N = 4$), isoproterenol (□; $N = 5$) and epinephrine (●, $N = 5$) on I_{sc} in *Necturus* urinary bladder. EC_{50} values indicated by arrows; see text.

TABLE II

Effect of α- and β-adrenoceptor antagonists on norepinephrine (10^{-5} M) stimulation of I_{sc} in Necturus urinary bladder

Antagonists added 20 min before NE (norepinephrine). I_{sc} recorded 30 min after NE. * P value for paired Student's t-test comparing pre- and norepinephrine treatment within the same tissue.

Treatment	I_{sc} ($\mu A/cm^2$)	P *
Control (12)	7.4 ± 1.2	-
NE alone (5)	16.3 ± 0.9	0.001
NE + 10^{-6} M propranolol (3)	14.9 ± 1.1	0.001
NE + 10^{-6} M phenoxybenzamine	11.3 ± 1.0	0.025

TABLE III

Cellular slope resistance $k\Omega \cdot cm^2$ after amiloride treatment (N = 8)

R_t	a	R'_t	a'	R_a	R'_a	R_b	R'_b	R_p
8.2±2.6	2.6	21.2±4.3	6.1	11.1±2.8	59.4±12.6	4.2±1.3	9.8±1.4	37.9±9.9

TABLE IV

Effect of norepinephrine (10^{-5} M) on transepithelial and cellular electrical properties of Necturus urinary bladders (N = 12)

Values in mV, $\mu A/cm^2$ and $k\Omega \cdot cm^2$.

	V_t	V_a	V_b	R_t	fR_a	I_{Na} *
Control	91.9±1.5	0.9±1.4	91.3±1.6	11.1±1.1	0.93±0.04	8.2±0.9
NOR	101.9±2.8	11.3±1.6	88.2±2.4	4.4±1.6	0.79±0.03	24.3±7.3

* $I_{Na} = I_{sc} - I'$ where $I' = 1.3 \pm 0.3$ $\mu A/cm^2$ after amiloride.

urinary bladder is mediated by an adrenergic receptor. The potency series (norepinephrine > epinephrine > isoproterenol) indicates that an α-adrenoceptor couples the agonist to Na^+ transport in the tissue [16].

Cellular effects of norepinephrine

Microelectrode studies were conducted in 'open-circuited' urinary bladders. Impaled urinary bladders were treated with either amiloride or norepinephrine. Voltage and fractional resistance measurements were not considered to be reliable for at least one minute after impalement [5].

In eight bladders receiving $5 \cdot 10^{-6}$ M amiloride, apical (V_a) and serosal (V_b) cell membrane voltage as well as fractional resistance (fR_a) were recorded (Figs. 6A,B). As V_t depolarized, both V_a and V_b hyperpolarized. fR_a increased. fR_a is inversely related to V_t before amiloride. fR'_a (after amiloride) bears no relation to the V_t before amiloride. Using R_t, R'_t, fR_0 and fR'_0, voltage dividers, a, were calculated. R_a, R_b and R_p in Table III were calculated using the method of Frömter and Gebler [5]. As indicated in the table, R_a and R_b were increased by amiloride.

Similar experiments with bladders receiving 10^{-5} M norepinephrine ($N = 12$) are summarized in Figs. 7 and 8 and Table IV. Transepithelial voltage and I_{sc} increase while R_t declines by 6.7 $k\Omega \cdot cm^2$ and fR_a decreases from 0.93 to 0.79. Apical membrane voltage (V_a) increases by 10.3 ± 0.6 mV as the basolateral membrane voltage (V_b) declines by 3.1 ± 0.6 mV. The time-course of fR_a decline is mirrowed by an increase in bladder I_{sc} (Fig. 7).

In eight bladders treated with norepinephrine (Table V), $5 \cdot 10^{-5}$ M amiloride depolarized V_t, decreased I_{sc}

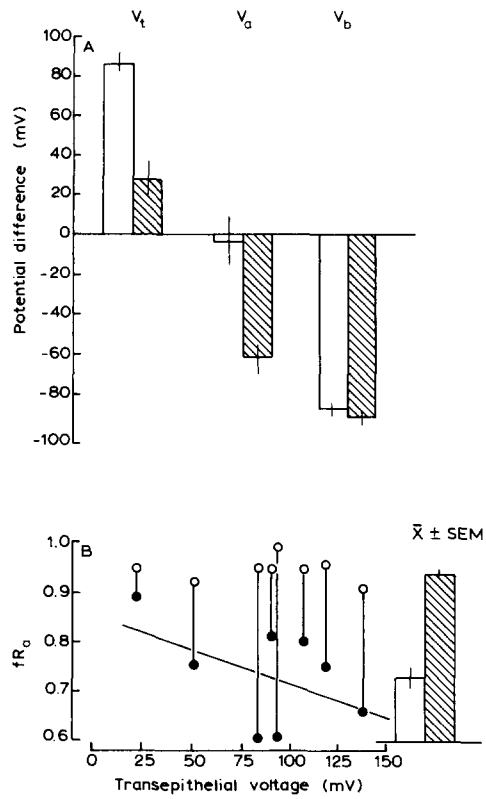

Fig. 6. Effects of $5 \cdot 10^{-6}$ M amiloride on the electrical properties of *Necturus* urinary bladder ($N = 8$). (A) Transepithelial (V_t), apical (V_a) and basolateral (V_b) before (open blocks) and after (shaded blocks) amiloride. (B) Apical membrane fractional resistance (fR_a) before (closed dots) and after (open dots) amiloride in relation to spontaneous open-circuit voltage (V_t).

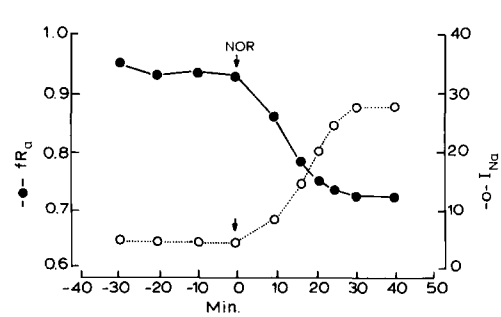

Fig. 7. Simultaneous changes in fR_a and I_{Na} affected by 10^{-5} M norepinephrine in a *Necturus* urinary bladder. Norepinephrine added at $t = 0$ min.

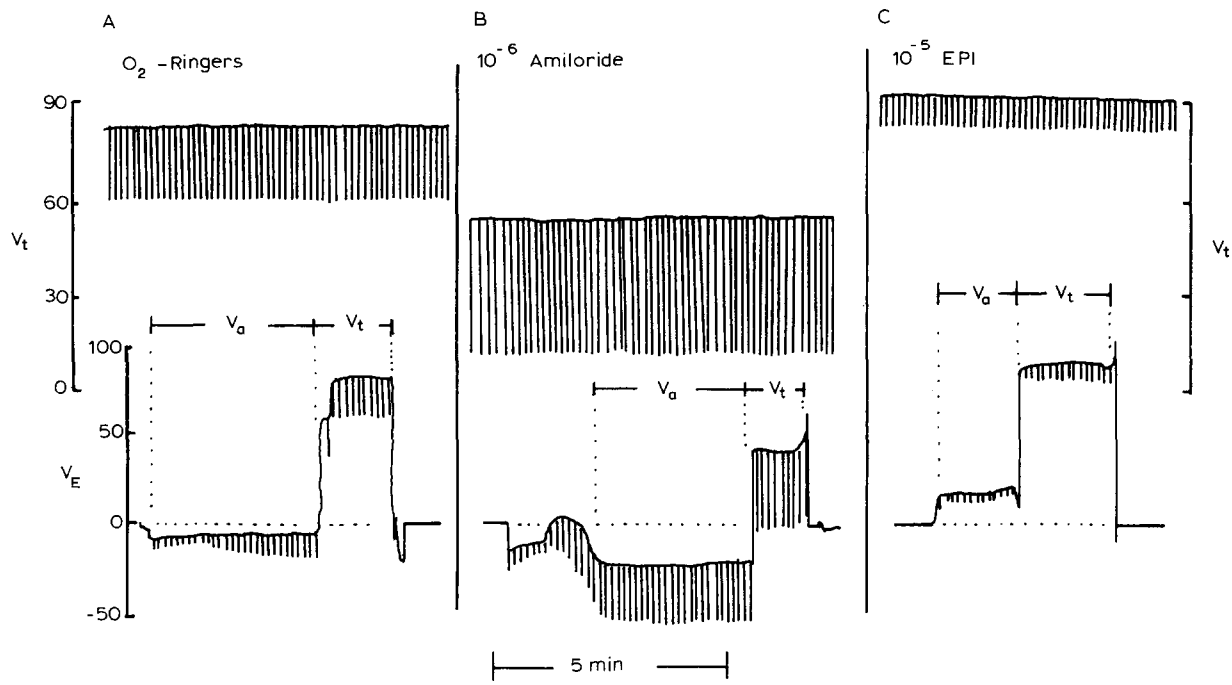

Fig. 8. Potential profile and resistance of a *Necturus* urinary bladder. The microelectrode is moved first from apical solution into the epithelial cell then across the basolateral membrane. (A) Spontaneous transepithelial (V_t) and microelectrode (V_e) potential. (B) Potentials following 1 μM amiloride. (C) Potentials following 10 μM norepinephrine. Current = 11.1 $\mu A/cm^2$.

and increased R_t. Initially V_a became -89.7 ± 3.5 mV as V_b hyperpolarized to -94.8 ± 2.3 mV ($V_t = 4.9 \pm 1.1$ mV). Over a 5 min period V_b subsequently depolarized to $91.9 \pm 1'.8$ mV. Amiloride increased both the fR_a to 0.97 ± 0.2 and the voltage divider ratio (a) from 4.1 ± 0.6 to 12.0 ± 0.8 (a'). Using fR_a and (a) before and after amiloride, R_a, R_b and R_p were calculated using the method of Frömter and Gebler [5]. Exposing norepinephrine-treated tissues to amiloride increase R_t, R_a and R_b but did not alter R_p (Table V). Norepinephrine applied to the serosal of the epithelium increases the conductances of both apical and basolateral cellular membranes. Consequently, the magnitude of the serosally directed I_{Na} is increased.

TABLE V

Transepithelial and cellular slope resistance following treatments with norepinephrine (N = 8)

After amiloride, $R'_t = 26.6 \pm 3.9$ k$\Omega \cdot cm^2$. Voltage divider before (a) 4.06 ± 0.82 and after (a') 12.0 ± 0.8 amiloride inhibition. Values in k$\Omega \cdot cm^2$. n.s., not significant below the 95% level by paired Student's t-test.

	Control	Norepinephrine	P (<)
R_t	12.7± 1.2	4.7±0.9	0.002
R_a	14.1± 1.7	2.4±0.6	0.001
$R_{a'}$	53.9± 9.3	48.1±4.8	n.s.
R_b	5.2± 1.0	2.8±0.5	0.01
R_p	54.5±10.4	59.1±8.3	n.s.

Discussion

Catecholamines such as epinephrine and norepinephrine are known to stimulate ion transport in other amphibian urinary tissues [17–19]. Heretofore no agent was known to stimulate Na^+ transport specifically in the mud puppy urinary bladder. In this in vitro study, we report that catecholamines stimulate amiloride-sensitive Na^+ transport in the bladder. Microelectrode assessment of cellular and paracellular resistances indicates that stimulation of ion transport occurs by a transcellular route. However, accurate measurement of transepithelial and cellular electrical properties is complicated by 'tissue' edge damage and microelectrode-induced membrane leakage. Edge-damage in a tight epithelium leads to underestimates of both transepithelial and cellular voltage and resistance by placing a low resistance shunt in parallel with the high-resistance cellular pathway [20,21]. Microelectrode impalement can introduce ion leaks in the cellular membrane [22] as well as large amounts of KCl into the cytoplasm [23]. We have gone to great effort to minimize these artifacts in the present study.

First, the Ussing chamber used in these experiments is designed to eliminate transepithelial artifacts occurring through edge-damage [3]. Since there is an inverse relationship between R_t and V_t (Fig. 2A) and a clear linear relationship between the logarithms of con-

ductance and I_{sc} (Fig. 2B), low resistance leaks make only minor contributions to the electrical behavior of the epithelium. Second, to avoid leaking K^+ into the impaled cell, only microelectrodes with high tip-resistances were used. Third, introduction of non-specific ion leaks into the apical membrane by microelectrode impalement is practically unavoidable. However, the magnitude of V_a, V_b, fR_a and a are high and very close to those estimated from impalements made through a 'stripped' serosal surface [5,7,11]. Consequently, microelectrode-induced artifacts appear to contribute minimally to our assessments of cellular parameters. However, these precautions notwithstanding, our R_a, R_b, fR_a and a values are likely to be slight underestimates.

Recent electrophysiological studies of amphibian tissue have established a framework for understanding the cellular mechanisms regulating active Na^+ transport in tight epithelia. The current view of transepithelial Na^+ transport in high-resistance epithelia originated with the proposal of Koefoed-Johnsen and Ussing [24]. Na^+ enters the epithelial cell by electrodiffusion across the apical membrane and is subsequently extruded through the basolateral membrane by the Na^+/K^+ pump. As as result of pump activity, K^+ accumulates within the cell. Cytoplasmic K^+ moves into the interstitium across the basolateral membrane by electrodiffusion. Since transepithelial flux involves a net flow of ions through the cytoplasm, the epithelium must maintain internal solutes and volume constant while engaging in transport. Intermembranous coordination of ion channels appears to be part of the regulatory mechanism maintaining ion and volume homeostasis as transport rates change [25]. The molecular mechanisms of channel regulation may be divided into four basic categories [26]: (1) control by transmembrane physical properties; (2) metabolic regulation by gene expression; (3) direct interaction of the channel with small ions; and (4) covalent modification by enzymatic activity. Of these, the latter two are most likely to participate in adrenoceptor regulation of Na^+ transport. Stimulation of adrenergic-dependent ion conductance with the epithelium probably relies upon the ability of certain inorganic ions and phosphorylating enzymes to activate protein ion channels in both the apical and the basolateral membrane.

However, there is crosstalk coordinating the conductances of the two membranes. Inhibition or activation of basolateral membrane K^+ conductance produces the same directional change in apical Na^+ conductance [11]. Several investigators have reported that the chemical potential for Na^+ diffusion across the apical cellular membrane changes with mucosal Na^+ concentration [6,7]. If the emf for Na^+ movement across the apical membrane is constant, variations in Na^+-dependent I_{sc} must be attributed entirely to changes in the conductance of the apical membrane. In 109 mM Na^+-Ringer's solution, the emf for Na^+ movement into the cell is expected to be about 70 mV [6]. Even when apical Na^+ concentrations are changed, the total thermodynamic driving force across the apical membrane may remain constant due to the 'self-regulation' of Na^+ permeability by Na^+ ions [7]. The polarity of V_a in *Necturus* urinary bladder following either amiloride or norepinephrine treatments can be correlated with the magnitude of R_a. As R_a increases following amiloride treatment, there is an increase in R_t and depolarization of V_t as V_a becomes more electronegative. On the other hand, as R_a and R_t decrease following norepinephrine treatment, both V_a and V_t become progressively more electropositive. As the rate of sodium movement across the apical membrane increases, the electrical current passing through the basolateral membrane (I_b) also increases. Earlier studies have shown that R_b increases simultaneously with R_a [5,11,12]. In the present study we found that R_b decreased when the I_{sc} was stimulated by norepinephrine (Fig. 8). In general, the K^+-dependent emf across the low-resistance basolateral membrane appears fairly constant [11]. Small changes in current passing through the membrane depolarize it away from the K^+-equilibrium potential. However, due to the comparatively small magnitudes of both the I_b and R_b, the depolarization is only a few millivolts. Thus the resistance of the apical membrane is pivotal in regulating the polarity of the cytoplasm as well as the rate of Na^+ transport. Since there do not appear to be voltage dependent conductances across either the apical or basolateral cell membranes [6,12], transmembrane physical properties appear to play a minor role in regulating transepithelial conductance.

The adrenal steroid aldosterone is a potent regulator of electrolyte balance in amphibian urinary epithelia [27]. The natriferic action of the hormone is brought about by altering gene expression and protein synthesis in target cells. A short-term effect of the hormone involves increasing apical membrane Na^+ permeability and enhancing the synthesis of Na^+/K^+-ATPase. Later events are associated with the synthesis of nascent apical membranes proteins which serve as ion channels [28]. Since steroid enhancement of Na^+ transport requires hours rather than minutes for activation, alteration of gene expression is not likely to occur as a result of exposure to norepinephrine.

In vitro, AVT increases water transfer through the skin and kidney of *Necturus* [1,29]. This 'pressor' substance will produce diuresis by increasing glomerular filtration in aquatic amphibia [30]. However, neither AVT, cAMP nor theophylline increases Na^+ transport in the isolated urinary bladder [2,3]. Either cAMP could not enter the cell or some other second messenger system couples the adrenergic receptor to various membrane channels. If the analog of cAMP used in these experiments cannot penetrate the cell membrane, the

adrenoceptor coupling mechanism could be adenylate cyclase-dependent. In such a case, increasing cAMP by either an AVT- or an adrenergic-coupled receptor system should increase the density of open Na^+ channels in the apical membrane [31,32]. Phosphorylation of the a subunit in epithelial cells by a cAMP-dependent protein kinase could serve to gate the Na^+ channel [33,34].

On the other hand, if cAMP does not play a role in this tissue, second-messenger coupling may be accomplished by either Ca^{2+} and protein kinase C or by changes in cytoplasmic pH. Diacylglycerol analogs known as tumor-promoting phorbol esters that activate protein kinase C also stimulate Na^+ transport in frog skin by selectively altering the conductance of the apical membrane [35]. In addition to activating protein kinase C, these substances increase cytoplasmic Ca^{2+} [36,37]. Cytosolic Ca^{2+}, in turn, activates basolateral K^+ conductance in a variety of epithelia [38–41]. Adrenoceptors coupled to cellular Ca^{2+} stores by phospholipase and protein kinase C may produce second messengers controlling both apical and basolateral membrane ion conductances.

Recent studies in frog skin suggest that cytoplasmic pH may regulate Na^+/K^+-ATPase activity, apical Na^+ and basolateral K^+ conductance in the frog skin [42]. As pH within the epithelial cell decreases, Na^+/K^+-ATPase activity declines and Na^+/H^+ exchange is stimulated across the basolateral membrane. Accumulation of Na^+ in the cytoplasm will influence the Ca^{2+}/Na^+ exchange in cellular membranes. If elevated pH persists, elevated Ca^{2+} levels will decrease K^+ permeability and Na^+-transport [38–42]. Although speculative, an α-adrenoceptor coupling to Na^+ transport by protein kinase, Ca^{2+} or pH provides an attractive explanation for the electrical response of the *Necturus* urinary bladder to norepinephrine.

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