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BARIUM INHIBITION OF SODIUM ION TRANSPORT IN TOAD BLADDER

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SUMMARY

The effect of Ba^{2+} on Na^+ transport and electrical characteristics of toad bladder was determined from change produced in short circuit current (I_{sc}), epithelial, apical and basal-lateral potentials (ψ_t, ψ_a, ψ_b), epithelial and membrane resistances (R_t, R_a, R_b) and shunt resistance (R_s). Mucosal Ba^{2+} had no effect. Serosal Ba^{2+} reduced I_{sc}, ψ_t, ψ_a, and ψ_b, but had no effect on R_t, R_a, R_b and R_s. Minimal effective Ba^{2+} concentration was $5 \cdot 10^{-5}$ M. The phenomenon was reversed by Ba^{2+} removal, but not by 86 mM serosal K^+. Ba^{2+} inhibition of I_{sc} did not impair the response to vasopressin which was quantitatively the same as controls. ψ_a with Ba^{2+} equalled ψ_b. After Ba^{2+} inhibition, ouabain produced no further decrease in ψ_t and I_{sc}. Ba^{2+} exposure after ouabain did not decrease ψ_t and I_{sc}. The results suggest that Ba^{2+} inhibits the basal-lateral electrogenic Na^+ pump.

INTRODUCTION

The isolated urinary bladder of the toad actively transports Na^+ from mucosal to serosal surface [1]. Na^+ transport creates an electrical gradient such that the mucosal solution polarity is negative to serosal solution. Both vasopressin and cyclic AMP increase Na^+ transport [2, 3]. Divalent cations are known to have significant effects on bladder transport. Calcium free serosal solution inhibits Na^+ transport [4], but high serosal Ca^{2+} concentration has no effect [5]. Manganese (10^{-4} M) inhibits transport as measured by short circuit current [6]. Barium has been shown to have a profound effect on cell membrane properties. K^+ conductivity is decreased in frog heart [7]. When Ba^{2+} is applied to the nutrient side of in vitro frog gastric mucosa, there is a marked increase in electrical resistance [8]. This is reversed by high concentrations of nutrient solution K^+. The phenomenon has been interpreted as Ba^{2+} inhibition of K^+ conductance, but this interpretation is complicated by the recent finding of an effect on Cl^- conductance also [9]. In frog muscle, Ba^{2+} reduces K^+ conductance by increasing the permeability ratio P_{Na}/P_K (10). P_K decreases and P_{Na} increases.

It seemed reasonable to predict that Ba^{2+} might have an inhibitory effect on

bladder Na^+ transport. Possible mechanisms by which this could occur include inhibition of the Na^+ pump at the basal-lateral surface, reduced permeability of the basal-lateral membrane to Na^+, or interference with Na^+ permeation of the apical membrane. The purpose of this investigation was fourfold: first to establish Ba^{2+} inhibition of Na^+ transport and study the effect on epithelial electrical resistances and potentials; second to determine the minimum Ba^{2+} concentration that effectively inhibits transport; third to measure the response of the Ba^{2+} inhibited bladder to vasopressin and high concentrations of serosal K^+, and finally to attempt to define the site of inhibition by microelectrode methods.

METHODS

Colombian *Bufo marinus* were obtained from Mogal-Ed Corp., Oshkosh, Wisc. They were kept at room temperature, given free access to water and fed with Tenebrio larvae. Two hemibladders from the same toad were removed after pithing the brain and spinal cord. They were mounted vertically in modified Ussing chambers with surface area of 3.14 cm^2. One was the experimental preparation and the other was a parallel control. Hemichambers were perfused with amphibian Ringers solution containing 100 mM Na^+, 4 mM K^+, 1.7 mM Ca^{2+}, 0.8 mM Mg^{2+}, 89 mM Cl^-, 18 mM HCO_3^-, 1 mM HPO_4^{2-} and 10 mM glucose. Circulation of solution was by gas lift bubbling with 95 % O_2/5 % CO_2. Ba^{2+} Ringers was made by substituting $BaCl_2$ for NaCl, and K^+ Ringer's by KCl substitution for NaCl. Osmolality of each solution was determined in an Advanced Osmometer and was 220±3 mosM/kg H_2O. Epithelial potential difference (ψ_t) was measured with a General Radio or Hewlett Packard voltmeter connected to two calomel electrodes which were introduced into the tubing-reservoir system of each hemichamber. Only bladders with potentials > 30 mV were employed. Current from an external source with microammeter was introduced into the system through electrolyte agar electrodes. Short circuit current (I_{sc}) was that density which reduced the epithelial potential to zero and represents active sodium transport [1]. I_{sc} was discontinued for a few seconds at periodic intervals to allow determination of ψ_t. After I_{sc} and ψ_t had been stable for 30 to 40 min, the mucosal hemichamber followed by the serosal hemi-chamber of the experimental preparation were washed 6 times with Ba^{2+} Ringers. The hemichambers of parallel controls were simultaneously washed with Ringers containing the same Na^+ concentration as the Ba^{2+} solution (choline substitution for Ba^{2+}). When I_{sc} and ψ_t had stabilized after Ba^{2+}, both hemichambers were washed 6 times with standard Ringers and recovery from Ba^{2+} was tested. The minimal effective Ba^{2+} concentration was determined from changes in I_{sc} and ψ_t after serosal exposure to 5 solutions (10^{-5} to 10^{-2} M). To test the effect of Ba^{2+} on K^+ conductance, bladders were first exposed to serosal 10^{-3} M Ba^{2+}. When ψ_t and I_{sc} stabilized, serosal solution was changed to 86 mM K^+, 10^{-3} M Ba^{2+}. The response of I_{sc} to vasopressin (Parke Davis, Co) was tested by addition of 2 units to the serosal solution after exposure to serosal Ba^{2+}, and was compared to the vasopressin response of parallel controls. Final vasopressin concentration was 130 munits/ml.

In microelectrode experiments, bladders were mounted horizontally in a modified Ussing chamber with mucosal surface upward. The mucosal hemichamber was open above to allow entrance of the microelectrode. Hemichamber volume was

2 ml. Ringers gassed with 95 % O_2/5 % CO_2 at 20 °C flowed through the lower closed serosal hemichamber by a siphon effect so as to keep the bladder firmly against a supporting tantalum mesh. Gassed Ringers flowed by gravity across the upper mucosal hemichamber. Flow of 2–3 ml/min was achieved by clamps on inflow and outflow tubing. Inflow was to the bottom of each hemichamber and outflow from the top. Serosal inflow was connected to 2 reservoirs (Ringers and 10^{-3} M Ba^{2+} Ringers). By clamp application and release, serosal solution could be changed by a rapid flow-through-system.

Microelectrodes were made from cleaned capillary glass tubing and pulled to a fine tip (< 1 μm) by a modification of the Nastuk glass puller. They were filled with methanol by gentle vacuum boiling. Methanol was displaced by distilled water, which in turn was displaced by filtered 3 M KCl. Tip resistance was 10–20 mΩ, and electrodes that had tip potentials > 3 mV were discarded.

Calomel electrodes were inserted into the outflow tubing of each hemichamber. The electrodes were connected to a Grass amplifier polygraph and to two microelectrode amplifiers. The microelectrode was connected to the microelectrode amplifiers by a KCl agar bridge, 3 M KCl reservoir and a calomel electrode. Outputs from the amplifiers were delivered to the polygraph. Thus continuous recordings of three potentials were obtained: epithelial (ψ_t), apical (ψ_a-microelectrode to mucosal solution), and basal-lateral (ψ_b-microelectrode to serosal solution).

Initially all bladders were bathed with Ringer's solution on both sides. When ψ_t became stable at > 20 mV, a bladder cell was impaled with the microelectrode. Impalement was accomplished with a Leitz micromanipulator under visualization through a Zeiss stereomicroscope with transmitted light. Cell polarity was always positive to mucosal solution and negative to serosal solution. Criteria for acceptable cell impalement were demonstration of $\psi_a = \psi_b$ and $\psi_a + \psi_b = \psi_t$. With Ringers on both sides of the bladder and with constant hydraulic force of serosal solution flow, preliminary experiments demonstrated that acceptable potentials could be recorded from a single cell impalement for 75–90 min. With a similar technique in frog skin, Cereijido and Curran have demonstrated stable potentials over a prolonged period following cell impalement [11]. After recording stable potentials, the serosal solution was changed from Ringers to 10^{-3} M Ba^{2+} Ringers. All 3 potentials decreased in 8–12 min. When potentials stabilized, Ba^{2+} serosal Ringer's was displaced by amphibian Ringers and reversibility of the Ba^{2+} effect was tested.

Epithelial resistance (R_t), apical resistance (R_a), basal-lateral resistance (R_b) and shunt resistance (R_s) were measured before and during serosal Ba^{2+}. Method B of Reuss and Finn [12] was employed. There is close correlation between values with this method and those obtained with cable analysis. The assumption is made that if mucosal Na^+ is replaced by K^+, R_a will increase but there will be no effect on R_b and R_s. The mucosal hemichamber inflow was connected to 2 gassed reservoirs (10^{-1} M Na^+ and 10^{-1} M K^+ Ringers). An external current source delivered 1 s pulses of 11.3 $\mu A \cdot cm^{-2}$ through silver-silver chloride electrodes. The shunt resistance can be calculated from

$$R_s = \frac{R_t \cdot R'_t \cdot (a'-a)}{R_t \cdot (a'+1) - R'_t \cdot (a+1)} \qquad (1)$$

R_t and a were determined with mucosal Na^+ Ringers. R_t was calculated from the voltage deflection ($\Delta\psi_t$) and current density. R_t', a and a' are defined in Table V. After calculation of R_s and experimental determination of R_t and R_a/R_b, apical and basal-lateral resistances were calculated from the equivalent circuitry of toad bladder.

$$R_t = \frac{(R_a+R_b)R_s}{R_a+R_b+R_s} \quad (2)$$

To determine if Ba^{2+} affected the ouabain sensitive electrogenic Na^+ pump in the basal-lateral membrane, I_{sc} and ψ_t were measured during a control period with Ringers, following serosal exposure to 10^{-3} M Ba^{2+} Ringers, and finally after serosal 10^{-3} M Ba^{2+}, 10^{-4} M ouabain. In a second series of experiments, the order was reversed and the serosal surface was exposed first to 10^{-4} M ouabain, and then to ouabain, Ba^{2+}.

RESULTS

Mucosal Ba^{2+} had no effect on I_{sc} and ψ_t. This was tested in 10 bladders at concentrations of 10^{-3} and 10^{-4} M. On the other hand, serosal 10^{-3} M Ba^{2+} had a significant effect. Table I shows that this concentration reduced I_{sc} from 12.8 to 7.5 $\mu A \cdot cm^{-2}$ and ψ_t from 39.3 to 23.5 mV. After removal of Ba^{2+}, there was good recovery, and potential and current returned to values comparable to control. Fig. 1 shows the concentration-response relationship of I_{sc} and ψ_t to 5 concentrations of serosal Ba^{2+}. 10^{-5} M concentration had no effect. At $5 \cdot 10^{-5}$ M Ba^{2+}, significant decrements of 19 % of both ψ_t and I_{sc} were observed. Inhibition of both parameters increased in a somewhat curvilinear fashion to 60 % of control at 10^{-2} M serosal Ba^{2+}.

Table II shows that the Ba^{2+} effect on I_{sc} and ψ_t was not reversed by high serosal $[K^+]$. I_{sc} and ψ_t of control and experimental hemibladders were comparable during the initial period. After standard Ringers was displaced with 1 mM Ba^{2+} Ringers in the experimental hemibladders, I_{sc} decreased from 15 to 8 $\mu A \cdot cm^{-2}$ and

TABLE I

RESPONSE OF SHORT CIRCUIT CURRENT AND EPITHELIAL ELECTRICAL POTENTIAL TO SEROSAL Ba^{2+}

I_{sc} = short circuit current. ψ_t = epithelial electrical potential. Experimental hemibladders were exposed to Ringers during control period, Ba^{2+} serosal Ringers during Ba^{2+} period and Ba^{2+} free serosal Ringers during recovery. Control hemibladders were not exposed to Ba^{2+}. Values are means ±1 S.E. of determinations in 10 bladders.

	$I_{sc}(\mu A \cdot cm^{-2})$			ψ_t(mV)		
	Control	10^{-3} M Ba^{2+}	Recovery	Control	10^{-3} M Ba^{2+}	Recovery
Control Hemibladder	11.1 ± 1.5	11.4 ± 1.6	11.9 ± 1.7	36.1 ± 3.8	38.7 ± 3.7	41.2 ± 3.0
Experimental Hemibladder	12.8 ± 1.3	7.5 ± 0.9	12.5 ± 1.5	39.3 ± 5.6	23.5 ± 4.4	31.9 ± 5.7

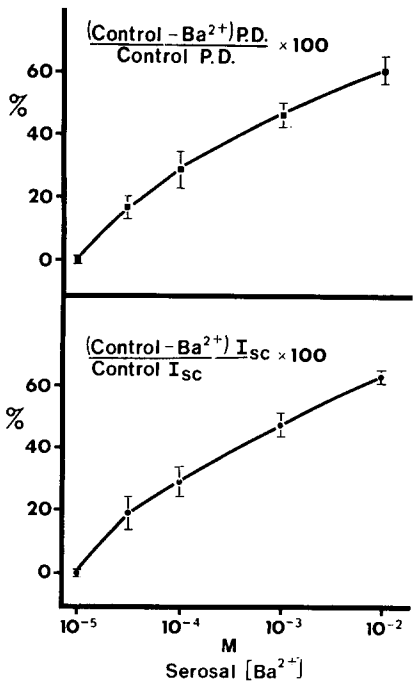

Fig. 1. Concentration-response relationship of I_{sc} and epithelial potential (P.D.) to serosal Ba^{2+}. Minimal effective concentration was $5 \cdot 10^{-5}$ M. Ten bladders tested at each of 5 concentrations. Data points are mean ± 1 S.E. of values of each concentration.

TABLE II

RESPONSE OF SHORT CIRCUIT CURRENT AND EPITHELIAL POTENTIAL TO SEROSAL Ba^{2+} AND TO SEROSAL Ba^{2+}, HIGH K^+

Experimental hemibladders exposed on serosal surface to Ringers, then to Ba^{2+} Ringers and finally to Ba^{2+}, high K^+ Ringers. Control hemibladders exposed to serosal Ringers throughout. Abbreviations defined in Table I. Values are means ± 1 S.E. of 9 experiments.

	I_{sc} ($\mu A \cdot cm^{-2}$)			ψ_t(mV)		
	Control	1 mM Ba^{2+}	1 mM Ba^{2+} 86 mM K^+	Control	1 mM Ba^{2+}	1 mM Ba^{2+} 86 mM K^+
Control Hemibladder	15.0 ± 3.1	13.0 ± 2.5	13.0 ± 2.1	44.0 ± 5.2	44.0 ± 4.3	44.0 ± 4.4
Experimental Hemibladder	15.0 ± 1.7	8.0 ± 0.9	8.0 ± 1.0	49.0 ± 2.3	32.0 ± 4.0	30.0 ± 3.3

TABLE III

RESPONSE OF SHORT CIRCUIT CURRENT TO VASOPRESSIN IN Ba^{2+} INHIBITED HEMIBLADDERS AND PAIRED CONTROLS

Experimental hemibladders exposed to serosal 10^{-2} M Ba^{2+} Ringers. Paired control hemibladders exposed to serosal Ba^{2+}- free Ringers. Serosal vasopressin concentration = 0.13 units/ml. Values are means ± 1 S.E. of 6 experiments.

	Pre vasopressin	I_{sc} ($\mu A \cdot cm^{-2}$) Post vasopressin	Δ (Post − Pre)
Control Hemibladder	38.7 ± 4.5	50.0 ± 5.1	12.0 ± 1.2
Experimental Hemibladder	22.8 ± 3.7	34.3 ± 4.7	12.0 ± 3.0

ψ_t from 49 to 32 mV. Displacement of the serosal Ba^{2+} Ringers by 1 mM Ba^{2+}, 86 mM K^+ Ringers had no appreciable effect. I_{sc} was 8.0 $\mu A \cdot cm^{-2}$ and ψ_t was 30 mV. Table III compares the response of I_{sc} to serosal vasopressin in control and paired experimental hemibladders inhibited by 10^{-2} M Ba^{2+}. Before vasopressin, control I_{sc} was 38.7 $\mu A \cdot cm^{-2}$ and Ba^{2+} inhibited I_{sc} was 22.8 $\mu A \cdot cm^{-2}$. After addition of vasopressin, control I_{sc} increased to 50 $\mu A \cdot cm^{-2}$ and Ba^{2+} inhibited I_{sc} increased to 34.3 $\mu A \cdot cm^{-2}$. Thus the vasopressin increment of 12 μA was the same in both control and Ba^{2+} treated bladders.

Table IV shows the microelectrode studies. Initially all bladders were bathed with standard Ringers on both surfaces. When epithelial potential stabilized, a selected bladder cell was impaled with the microelectrode. Cell polarity was negative to serosal solution and positive to mucosal solution. Similar cell polarity has been demonstrated by Frazier [13, 14] and Chowdhury and Snell [15]. The mean basal-lateral membrane potential of 15 mV was not significantly different from the mean apical membrane po-

TABLE IV

EFFECT OF SEROSAL Ba^{2+} ON EPITHELIAL, APICAL AND BASAL-LATERAL BLADDER POTENTIALS

ψ_a = apical potential. ψ_b = basal-lateral potential. ψ_t = epithelal potential. ψ_a and ψ_b measured by microelectrode impalement during control period, after serosal exposure to Ba^{2+} Ringers and during recovery period after washout of serosal Ba^{2+}. Values are means ± 1 S.E. of studies in 16 bladders.

	Control (mV)	10^{-2}M Ba^{2+} (mV)	Δ (Control − Ba^{2+}) (mV)	Recovery (mV)
ψ_a	16.0 ± 2.8	6.6 ± 1.5	9.4 ± 1.4	15.0 ± 3.7
ψ_b	15.0 ± 2.0	5.5 ± 1.0	9.1 ± 1.4	11.0 ± 2.0
ψ_t	33.0 ± 5.0	14.0 ± 2.7	18.9 ± 2.7	30.0 ± 3.5

TABLE V

EFFECT OF SEROSAL Ba^{2+} ON BLADDER RESISTANCES

Resitance measurements by method B of Reuss and Finn [12]. R_t = epithelial resistance. R_s = shunt resistance. R_a = apical resistance. R_b = basal-lateral resistance. $a = R_a/R_b = \Delta\psi_a/\Delta\psi_b$ with 1 s current pulse of 11.3 $\mu A \cdot cm^2$. R'_t and a' = values determined with mucosal K^+ Ringers. Values are means ± 1 S.E. and are given in ohm · cm^2. Numbers in parentheses signify the number of experiments.

	a	a'	R_t	R'_t	R_a ohm · cm^2	R_b	R_s
Control	1.66 ±0.14 (20)	5.40 ±0.69 (5)	3244 ±167 (43)	5115 ±311 (5)	3390 ±240 (5)	2379 ±217 (5)	10172 ±1712 (5)
10^{-2} M Ba^{2+}	1.65 ±0.13 (20)	5.24 ±0.31 (10)	3566 ±143 (43)	5363 ±156 (10)	3836 ±366 (10)	2254 ±122 (10)	10513 ±1188 (10)

tential of 16 mV. The sum of the apical and basal potentials equalled epithelial potential. When serosal solution was changed to 10^{-2} M Ba^{2+} Ringers, mean epithelial potential fell from 33 to 14 mV. Simultaneously mean apical potential decreased to 6.6 mV and basal-lateral potential to 5.5 mV. The mean decrease of apical potential ($\Delta\psi_a$) was 9.4 mV±S.E. of 1.4. The mean decrease of basal-lateral potential ($\Delta\psi_b$) was 9.1 mV±S.E. of 1.4. These values were not appreciably different and mean $\Delta\psi_a/\Delta\psi_b$ was 1.2±S.E. of 0.2, a value reasonably close to unity. When the Ba^{2+} serosal solution was displaced by standard Ringers, there was almost complete recovery of the 3 potentials. Values during the recovery period were comparable to initial control determinations.

The resistance studies are summarized in Table V. Ba^{2+} did not change epithelial resistance which was 3566±143 ohm · cm^2 compared to control of 3244±167. The ratio of apical to basal-lateral resistance was 1.66±0.14 in control and 1.65±0.13

TABLE VI

EFFECT OF SEROSAL Ba^{2+} FOLLOWED BY Ba^{2+}+OUABAIN ON ψ_t AND I_{sc}

Serosal surface of bladder first exposed to Ba^{2+} and then to Ba^{2+}+ouabain. Abbreviations defined in Table I. Values are means ±1 S.E. of 7 experiments.

	Ringers		10^{-2} M Ba^{2+}		10^{-2} M Ba^{2+} + 10^{-4} M ouabain	
	ψ_t (mV)	I_{sc} ($\mu A \cdot cm^{-2}$)	ψ_t (mV)	I_{sc} ($\mu A \cdot cm^{-2}$)	ψ_t (mV)	I_{sc} ($\mu A \cdot cm^{-2}$)
Control	47.5 ±10.5	31.2 ±8.7	49.8 ±9.7	31.6 ±8.2	49.4 ±9.4	32.1 ±8.1
Experimental	60.5 ±6.9	22.9 ±3.9	32.6 ±5.1	11.2 ±1.5	28.6 ±3.5	11.36 ±1.65
P (ψ_t)				> 0.5		
P (I_{sc})				> 0.9		

TABLE VII

EFFECT OF SEROSAL OUABAIN FOLLOWED BY OUABAIN + Ba^{2+} ON ψ_t AND I_{sc}

Serosal surface of bladder first exposed to ouabain and then to ouabain + Ba^{2+}. Abbreviations defined in Table I. Values are means ± 1 S.E. of 7 experiments.

	Ringers		10^{-4} M ouabain		10^{-4} M ouabain + 10^{-2} M Ba^{2+}	
	ψ_t (mV)	I_{sc} ($\mu A \cdot cm^{-2}$)	ψ_t (mV)	I_{sc} ($\mu A \cdot cm^{-2}$)	ψ_t (mV)	I_{sc} ($\mu A \cdot cm^{-2}$)
Control	43.0 ± 7.9	20.9 ± 3.8	45.1 ± 7.5	18.8 ± 2.6	43.4 ± 8.8	15.8 ± 1.5
Experimental	53.7 ± 4.8	20.2 ± 3.8	27.6 ± 9.2	7.1 ± 3.7	13.2 ± 5.3	4.3 ± 2.4
$P(\psi_t)$				0.1		
$P(I_{sc})$				0.5		

with barium, values which were not statistically different. Ba^{2+} did not produce any significant changes in apical, basal-lateral and shunt resistances. Mean R_a ± 1 S.E. was 3390 ± 240 ohm · cm^2 in control and 3836 ± 366 with Ba^{2+}. Mean R_b was 2379 ± 217 ohm · cm^2 in control and 2254 ± 122 with Ba^{2+}. Control R_s was 10 172 ± 1712, and with Ba^{2+} was 10 513 ± 1188 ohm · cm^2.

Table VI and VII summarize the ouabain and Ba^{2+} studies. Data for the experimental and parallel control hemibladders is given. In Table VI, exposure of the serosal surface to 10^{-2} M Ba^{2+} reduced I_{sc} from 22.9 to 11.2 $\mu A \cdot cm^{-2}$ and epithelial potential from 60.5 to 32.6 mV. After subsequent exposure to serosal 10^{-4} M ouabain and 10^{-2} M Ba^{2+}, I_{sc} was 11.36 $\mu A \cdot cm^{-2}$ and potential was 28.6 mV. These values were not statistically different from those obtained with Ba^{2+} alone. P value for the difference was > 0.9 for I_{sc} and > 0.5 for potential. Table VII shows data when the order of exposure was reversed. The bladder was exposed first to serosal ouabain and then to ouabain + Ba^{2+}. Ouabain reduced mean I_{sc} from 20.2 ± 3.8 $\mu A \cdot cm^{-2}$ to 7.1 ± 3.7, and potential from 53.7 ± 4.8 mV to 27.6 ± 9.2. When bladders were subsequently exposed to ouabain + Ba^{2+}, I_{sc} was 4.3 ± 2.4 $\mu A \cdot cm^{-2}$ and potential was 13.2 ± 5.3 mV. These values were not statistically different from those with ouabain alone. P values for the differences were > 0.5 for I_{sc} and > 0.1 for potential. Thus ouabain did not produce further reduction of ψ_t and I_{sc} after Ba^{2+}, and Ba^{2+} did not reduce ψ_t and I_{sc} further after ouabain.

DISCUSSION

Toad bladder transports sodium from mucosal to serosal fluid. Na^+ penetration of apical membrane is a partially passive process which is independent of ouabain [16] and occurs down an electrochemical gradient [17]. With low mucosal [Na^+] or low serosal [K^+], sodium flux from mucosal fluid into cell becomes ouabain sensitive and dependent on the activity of the basal-lateral electrogenic pump [16]. The latter actively transports Na^+ up an electrochemical gradient from cellular pool to serosal fluid [17]. In open circuit bladder, cell polarity is positive to mucosal fluid [13–15]

which suggests that Na^+ permeability of apical membrane is greater than Cl^- permeability with consequent separation of charge. Apical membrane functions as a sodium sensitive electrode in toad bladder [18] and also in frog skin [19]. Sodium permeability of apical membrane is increased by vasopressin and cyclic 3',5'-AMP [20–22], with resultant augmented epithelial transport. The apical and basal-lateral membranes act as passive electrical resistors (R_a and R_b) in series. They are in parallel with the shunt resistance (R_s) which is presumably located in the zona occludens. R_a is about 3800 ohm · cm^2, R_b 2800 ohm · cm^2 and R_s 12 000 ohm · cm^2 [12].

Ba^{2+} depresses equally both short circuit current and epithelial potential when present in serosal solution. There is no effect from mucosal Ba^{2+}. Unlike other tissues [7, 8, 10], there is no effect on resistances, and apical, basal-lateral, shunt and epithelial resistances were unchanged. The decrease in epithelial potential was the result of equal decreases of apical and basal-lateral potentials. Minimal effective concentration of Ba^{2+} was $5 \cdot 10^{-5}$ M, which decreased epithelial potential and I_{sc} by 19 %. The phenomenon is reversible and upon withdrawal of serosal Ba^{2+} there was recovery of short circuit current and potentials. However, depression of the electrical parameters was not reversed by the addition of a high K^+ concentration in the presence of Ba^{2+}. The I_{sc} response to vasopressin was not depressed by Ba^{2+}. It was quantitatively identical in control and Ba^{2+} inhibited bladders. Following Ba^{2+} exposure, ouabain did not produce further decrease in epithelial potential and I_{sc}. Following ouabain, Ba^{2+} did not depress the electrical parameters further.

The experimental results give no support to an action of Ba^{2+} on apical membrane. If Ba^{2+} had an apical effect, it would presumably be to decrease Na^{2+} permeability. As apical membrane functions as an Na^+ electrode, an apical effect of Ba^{2+} should have been accompanied by an increase in R_a which did not occur. Also against an apical effect was the response to vasopressin, an agent known to affect apical permeability [20–22]. Finally, the lack of effect of mucosal Ba^{2+} is against an apical membrane effect. Agents such as amiloride with a known apical effect [20, 23] are primarily active when in mucosal fluid. Another possible site of Ba^{2+} effect could be the zona occludens. This is normally high resistance (R_s), and R_s can be increased by low mucosal [Na^+] and decreased by hyper-osmolality of mucosal fluid [24]. If Ba^{2+} decreased R_s, with no effect on R_a, R_b and the electrogenic pump, a back leak of Na^+ from serosal to mucosal fluid could occur with the net result of decreased ψ_t, ψ_a, ψ_b and I_{sc}. As there was no significant change of R_s with Ba^{2+}, this possibility can be excluded.

The experimental evidence points strongly to the basal-lateral membrane as the site of action of Ba^{2+}. The actual site is more likely to be the electrogenic pump rather than the membrane per se. The basal-lateral membrane was originally regarded as a K^+ sensitive electrode [18]. However, there have been subsequent modifications. A significant basal-lateral potential remains after serosal [K^+] has been increased to a concentration equal to that in the cell. The residual potential is believed to be the result of activity of the electrogenic Na^+ pump [14, 17]. Thus basal-lateral membrane probably has both K^+ and Na^+ conductance, which are not mutually dependent. If Ba^{2+} lowered either or both conductances an increase in R_b would be expected, and this did not occur. A selective effect on K^+ conductance should be reversed by high serosal [K^+] as in other tissues [7, 8, 10] with restoration of epithelial potential. As 86 mM serosal K^+ did not reverse the Ba^{2+} effect on potential and I_{sc}, it seems unlikely that Ba^{2+} had a selective action on basal-lateral K^+ conductance.

The actual site of the Ba^{2+} effect is most likely the basal-lateral electrogenic pump. Ouabain is an established inhibitor of the pump [25–27]. Although not definitive, the failure of Ba^{2+} to produce further decrease of I_{sc} and potential after ouabain and the failure of ouabain to reduce I_{sc} and potential after Ba^{2+} (Tables VI and VII) is strongly suggestive of pump inhibition. Some or part of the electrogenic pump may be transport ATPase. Ouabain inhibition of ATPase may be reversed by a high $[K^+]$ [28]. Some of the inhibition of bladder I_{sc} by ouabain may be reversed by 14 mM K^+ serosal fluid [26]. However, 86 mM serosal K^+ failed to reverse the Ba^{2+} effect. Thus Ba^{2+} inhibition of the electrogenic pump must occur at a site different to that of ouabain. The interpretation of Ba^{2+} inhibition of the electrogenic pump would adequately explain the experimental findings. The basal-lateral potential is most likely an EMF generated by the active Na^+ pump. Current flow (I) would also be generated by the pump, and the apical potential is most likely current flow across a resistor (IR drop). Thus as no resistance changes occurred from exposure to Ba^{2+}, the decrease of epithelial, apical, basal and shunt potentials (in bladder equivalent circuitry, $\psi_t = \psi_a$) could be accounted for by inhibition of the electrogenic pump and decrease of I.

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