Action of Ouabain on Sodium

Transport in Toad Urinary Bladder

Evidence for Two Pathways for Sodium Entry

ARTHUR L. FINN

From the Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514

ABSTRACT   The cardiac glycoside ouabain inhibits transepithelial sodium transport in the toad urinary bladder. It is shown that this drug reduces the rate coefficient for sodium exit at the serosal pump site. In addition, ouabain inhibits entry across the mucosal border whenever the electrochemical potential gradient for sodium is made less favorable. The data are interpreted as indicating the existence of two separate pathways for sodium entry, one of which is ouabain inhibitable.

In the isolated toad urinary bladder and frog skin, sodium carries all of the current across the tissue when it is clamped at zero potential in the absence of a chemical gradient across the system (Leaf et al., 1958; Ussing and Zerahn, 1951). Most of this active sodium transport is inhibited by such cardiac glycosides as ouabain (Herrera, 1968); it has been assumed, though never directly demonstrated, that this substance acts solely at the serosal transport step, where sodium movement is clearly against an electrochemical potential gradient (Frazier and Leaf, 1963). However, ouabain does not simply inhibit the serosal sodium pump, for we have shown (Finn, 1973) that there is in the presence of ouabain a potassium-dependent potassium efflux mechanism at the serosal border which is inhibited by furosemide; furthermore, in frog skin Biber (1971) has shown that ouabain inhibits sodium influx into the frog skin when the outside sodium concentration is reduced. The present studies were undertaken to explore the mechanism of action of ouabain further.

MATERIALS AND METHODS

The toads used in these studies were *Bufo marinus* of Colombian origin, and were obtained from the Pet Farm, Miami, Florida or from the Tarpon Zoo, Tarpon Springs, Florida. The toads were pithed, and the bladder was removed and placed in Ringer

solution, composed of (mM) NaCl 109, KCl 2.5, CaCl₂ 0.9, NaHCO₃ 2.4, and glucose 5.6. Solutions were gassed with room air and had a pH of about 7.8. When the sodium concentration was changed it was replaced mole-for-mole with choline chloride (recrystallized from hot ethanol). When the potassium concentration was changed, it was exchanged for sodium on a mole-for-mole basis. The bladders were mounted between halves of a Lucite chamber and were kept short-circuited throughout all experiments except as otherwise described. Sodium washout studies were subsequently performed, as previously described (Finn and Rockoff, 1971). Briefly, tracer (^{24}Na or ^{22}Na) is added to the mucosal medium and allowed to remain in the chamber for at least 45 min, during which nonradioactive Ringer is continually pumped through the serosal chamber. At the conclusion of this loading period, nonradioactive Ringer is pumped through both chambers at a (nominal pump) rate of 38.2 ml/min per chamber for 1 min, in order to remove all of the loading solution from the chamber. The flow rate is then abruptly reduced to 7.8 ml/min per chamber, and all effluent is collected in test tubes mounted in a fraction collector.

The time-course of tracer appearance in each medium is fitted simultaneously with linear combinations of exponentials with the use of a digital computer program called "simulation analysis and modeling" (SAAM) (Berman et al., 1962). From this fitting procedure, k_{S1}, k_{M1}, and J_{1M} (symbols are defined in Table I) are directly obtained. From J_{1M} and J_{net}, the short-circuit current (in bladders clamped at zero PD), we calculate J_{M1}, assuming a steady state for sodium. Then,

$$J_{M1}/k_{M1} = A_1,$$

$$A_1 \cdot k_{S1} = J_{S1},$$

and

$$J_{S1} - J_{net} = J_{1S}.$$

If, however, there is either a transepithelial potential or chemical gradient, there is no way to determine J_{net}. Thus only mucosal influx and the two efflux rate coefficients can be determined under such circumstances (vide infra). The theoretical considerations and methods of calculation have been described in detail by Finn and Rockoff (1971).

In each case, an appropriate control study was performed, and then ouabain was added to the serosal medium at a final concentration of 5×10^{-4} M; the inhibitor was present for at least 90 min before a subsequent washout study was performed. No experiment was carried out unless the initial transepithelial potential difference was 50 mV or higher.

RESULTS

Effect of Ouabain on Steady-State Sodium Kinetics

The mean stable short-circuit current 90 min after ouabain addition was 15.7 ± 5.5 (SEM)% of the control value. This level was reached in about 45 min, and remained constant throughout the second washout study. As shown,[1]

[1] For all of the experiments in this paper, only the faster of the two tissue sodium compartments, compartment 1, was affected.

TABLE I

EFFECT OF OUABAIN ON SODIUM KINETICS IN BLADDERS MOUNTED IN RINGER SOLUTION

	J_{1M}		J_{M1}		A_1		J_{81}		J_{1S}		k_{M1}		k_{S1}		J_{Na}^{net}	
	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O
	2.49	2.52	2.20	2.50	6.60	5.38	0.359	0.059	0.068	0.042	0.333	0.465	0.054	0.011	0.291	0.017
	3.78	5.16	3.06	5.11	15.56	17.25	0.764	0.065	0.048	0.018	0.197	0.296	0.049	0.004	0.716	0.047
	5.59	4.44	5.04	4.38	13.21	17.18	0.671	0.515	0.114	0.452	0.381	0.255	0.051	0.030	0.557	0.063
	3.86	5.50	3.40	5.47	11.25	16.91	0.582	0.049	0.119	0.014	0.302	0.323	0.052	0.003	0.463	0.035
	1.85	2.02	1.62	1.93	8.48	5.65	0.713	0.429	0.476	0.336	0.191	0.342	0.084	0.076	0.237	0.094
	3.67	2.34	3.44	2.28	11.96	7.80	0.316	0.132	0.082	0.077	0.287	0.293	0.026	0.017	0.234	0.055
Mean ± SEM	3.54 ±0.53	3.66 ±0.50	3.12 ±0.48	3.61 ±0.64	11.18 ±1.32	11.69 ±2.45	0.567 ±0.077	0.208 ±0.085	0.151 ±0.066	0.157 ±0.077	0.282 ±0.031	0.329 ±0.030	0.053 ±0.008	0.023 ±0.011	0.416 ±0.080	0.052 ±0.011
ΔC-O	−0.12 ±0.50		−0.49 ±0.55		−0.51 ±1.59		0.359 ±0.087		−0.005 ±0.070		−0.048 ±0.042		0.029 ±0.008		0.365 ±0.083	
P difference	NS		NS		NS		<0.01		NS		NS		<0.02		<0.01	

Each bladder was studied before and at least 90 min after the addition of ouabain to the serosal solution. All data are expressed as mean ± SEM. (C = control, O = ouabain.) J_{ij} = flux into compartment i from compartment j, $\mu eq \cdot min^{-1} \cdot 100$ mg dry weight^{-1}. A_1 = compartment size, $\mu eq \cdot 100$ mg dry weight^{-1}, k_{ij} = rate coefficient into compartment i from compartment j, min^{-3}. ΔC-O = difference between control and ouabain-treated preparations.

Compartment 1 is the transport pool (see Finn and Rockoff, 1971). The slow compartment is not shown here, but is not affected by ouabain.

ouabain produced a significant decrease in the efflux of sodium from the cells to the serosal solution (J_{s1}, the "pump flux") and in the rate coefficient at the same site (k_{s1}, the "pump rate coefficient"). However, there was no significant change in any of the other kinetic parameters. In particular, the size of the transport pool was not changed, nor were the fluxes at the mucosal border of the pool.

This result was somewhat surprising, since it had previously been shown that ouabain produced a rise in the sodium content of both whole toad bladders (Herrera, 1968), and isolated epithelial cells (MacKnight et al., 1971). However, if the passive backflux of sodium across the serosal border is small enough, the internal sodium concentration might not change if the efflux mechanism is only partly inhibited. Furthermore, ouabain might affect an uptake pathway at the mucosal border as in frog skin (Biber, 1971). Experiments were therefore performed in which the mucosal sodium concentration was reduced to 2.4, 10, or 20 mM. As shown in Table II, there was in each

TABLE II

EFFECT OF OUABAIN ON SODIUM KINETICS IN THE PRESENCE OF A LOW MUCOSAL SODIUM CONCENTRATION

Na$_M$	Control			Ouabain		
	J_{1M}	k_{M1}	k_{S1}	J_{1M}	k_{M1}	k_{S1}
2.4	0.152	0.220	0.230	0.089	0.211	0.076
2.4*	0.089	0.320	0.125	0.042	0.226	0.031
10.0*	1.511	0.191	0.146	0.877	0.128	0.016
10.0*	3.121	0.271	0.067	0.344	0.115	0.007
10.0	0.498	0.193	0.102	0.372	0.285	0.014
10.0	2.991	0.430	0.060	1.881	0.311	0.023
20.0	3.171	0.422	0.038	1.837	0.320	0.024
20.0	1.587	0.224	0.137	0.785	0.336	0.017
Δ ouabain, %				−41.4	−4.1	−67.0
				−52.8	−29.4	−75.2
				−42.0	−33.0	−89.0
				−89.0	−57.6	−90.0
				−25.3	+47.7	−86.3
				−37.1	−27.7	−61.7
				−42.1	−24.2	−36.8
				−50.5	+50.0	−87.6
Mean % change				−47.5±6.6	−9.8±13.8	−74.1±6.5
P				<0.001	NS	<0.001

Na$_M$ = mucosal sodium concentration in both control and ouabain washout studies. Other abbreviations as in Table I.
Because of the variation in J_{1M} and k_{S1} expected from the differences in Na$_M$ (Finn, 1971), differences between control and ouabain-treated preparations all given as percent change.
* In addition to the data shown in those experiments indicated by an asterisk (*), a separate control study was performed with normal Ringer solution in both bathing media (see text).

case a marked inhibitory effect of ouabain on the influx of sodium across the mucosal border, J_{1M}, and on the pump rate coefficient, k_{S1}, thus confirming the suggestion of at least two sites of action of this inhibitor.

Several additional comments about this table are in order. First, pool sizes were not determined because the sodium concentration was lowered on the mucosal side only. Under these conditions, the short-circuit current is not an accurate measure of net sodium transport. However, as shown previously (Finn and Rockoff, 1971), determination of mucosal sodium entry, J_{1M}, and both efflux rate coefficients, k_{M1} and k_{S1}, depend only on the establishment of a steady state for tracer at the start of the washout. Second, J_{1M} is lower in these studies than in normal Ringer, as expected, although there is considerable variation. In addition to the measurements shown, sodium kinetics were determined on three of the bladders with Ringer solution ($Na_M = Na_S = 111.4$) in both sides before the reduction in Na_M. The values obtained were similar to those reported previously and to those shown in Table I ($J_{1M} = 4.93$, 4.85, and 6.91, respectively, $A_1 = 10.43$, 12.51, and 22.16, respectively, and $k_{S1} = 0.032$, 0.080, and 0.053, respectively). Third, the values for k_{S1} in the low sodium controls are higher than in 111.4 Na, and tend to decrease as Na_M increases, as previously described (Finn, 1971).

Finally, it should be added that these changes are not simply due to the passage of time, although it is unfortunate that the ouabain effects cannot be reversed. Thus, in previous experiments, in which either vasopressin was added (Finn, 1971) or the serosal potassium concentration was varied (Finn and Hutton, 1974), randomization of the order in which the studies were done did not alter the results. Furthermore, repeated determinations in the same tissue under control conditions yield results which are not significantly different from one another (Finn, unpublished data).

Effect of Changes in Potassium Concentration on Ouabain-Sensitive Sodium Influx

We next explored the effects of ouabain on sodium fluxes in a low potassium medium. As shown in Table III, there was a $38.8 \pm 7.5\%$ decrease in mucosal sodium influx, J_{1M}, under these conditions. It should be noted that the effect of ouabain on the pump rate coefficient is no longer significant. This is not unexpected, since we have previously shown (Finn and Hutton, 1974) that reduction of the potassium concentration alone has an inhibitory effect on the pump rate coefficient. Furthermore, in these studies the sodium pool, initially elevated owing to the low K_S (Finn and Hutton, 1974), decreased, presumably due to the decrease in net entry.

As a result of the decrease in pool size, mucosal sodium efflux also decreased significantly. That this is not a direct effect of ouabain on efflux is indicated by the failure to see a significant effect on k_{M1}. It should be added that there is no evidence in toad urinary bladder for sodium-sodium exchange either

TABLE III

EFFECT OF OUABAIN ON SODIUM KINETICS IN THE PRESENCE OF LOW SEROSAL POTASSIUM

	J_{1M}		J_{M1}		A_1		J_{S1}		J_{1S}		k_{M1}		k_{S1}		J_{Na}^{net}	
	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O
	7.02	3.16	6.98	3.16	23.00	16.66	0.460	0.233	0.422	0.233	0.304	0.190	0.020	0.014	0.038	0
	6.08	5.04	6.05	5.04	15.52	14.95	0.186	0.090	0.157	0.090	0.390	0.338	0.012	0.006	0.029	0
	4.88	3.89	4.80	3.89	14.55	9.43	0.698	0.226	0.617	0.226	0.330	0.418	0.048	0.024	0.081	0
	4.28	3.37	4.16	3.32	14.72	12.95	0.339	0.298	0.223	0.252	0.283	0.257	0.023	0.023	0.116	0.046
	6.79	2.66	6.63	2.65	19.63	11.52	0.491	0.138	0.331	0.128	0.338	0.230	0.025	0.012	0.160	0.010
	10.82	4.90	10.68	4.88	22.22	13.50	0.200	0.148	0.058	0.132	0.481	0.362	0.009	0.011	0.142	0.016
Mean ± SEM	6.64 ±0.94	3.84 ±0.39	6.55 ±0.94	3.82 ±0.39	18.28 ±1.56	13.17 ±1.04	0.396 ±0.080	0.189 ±0.031	0.301 ±0.082	0.177 ±0.028	0.354 ±0.029	0.299 ±0.036	0.023 ±0.006	0.015 ±0.003	0.094 ±0.022	0.012 ±0.007
ΔC-O	2.81 ±0.87		2.73 ±0.86		5.11 ±1.36		0.207 ±0.072		0.124 ±0.070		0.055 ±0.032		0.008 ±0.004		0.082 ±0.020	
P difference	<0.05		<0.05		<0.02		<0.05		NS		NS		NS		<0.01	

C = control (K_S = 0.5 mM), O = ouabain. Other abbreviations as in Table I. Each bladder was mounted in Ringer solution and short-circuited. Serosal K was then reduced to 0.5 mM. After stabilization of the short-circuit current at a new (lower) value, a wash-out study was performed. Subsequently ouabain was added and a second wash performed at least 90 min later.

in the absence (Finn and Hutton, 1974) or in the presence (Finn, unpublished observations) of ouabain. Since the mucosal solution sodium "pool" does not change when ouabain is added, the sequence of events almost certainly is that ouabain first inhibits both unidirectional entry, J_{1M}, and pump rate coefficient k_{S1} (and hence pump flux J_{S1}). Since the former is the much larger of the two affected fluxes, the pool falls, thus decreasing J_{M1}, the measured efflux. When a steady state is reestablished, the net fluxes at the two borders are once again equal to one another.

Because of these results it seemed reasonable to test whether or not a rise in potassium levels in the medium, and hence in the cells, could prevent the effect of ouabain on sodium influx in a low sodium medium. Therefore, as shown in Table IV, four bladders were studied in which the potassium

TABLE IV

EFFECT OF OUABAIN ON SODIUM KINETICS IN LOW Na, HIGH K MEDIA

	10 K, 10 Na			10 K, 10 Na + Ouabain		
	J_{1M}	k_{M1}	k_{S1}	J_{1M}	k_{M1}	k_{S1}
	0.732	0.348	0.049	0.771	0.416	0.028
	0.367	0.205	0.107	0.291	0.246	0.036
	0.426	0.351	0.079	0.615	0.387	0.051
	1.102	0.398	0.094	1.073	0.377	0.036
Mean ± SEM	0.657	0.326	0.082	0.688	0.356	0.038
	±0.169	±0.042	±0.012	±0.163	±0.038	±0.005
Δ ouabain				−0.031	−0.031	0.044
				±0.058	±0.019	±0.012
P				NS	NS	<0.05

concentration of the serosal solution was raised to 10 mM. As we have previously shown (Finn, 1973) a rise in the serosal potassium concentration of this magnitude results in a rise in the size of the potassium transport pool. As shown in the table, ouabain caused a decrease in the pump rate coefficient, but there was no effect on mucosal entry, even when Na_M is reduced. Thus, ouabain inhibits sodium entry when either mucosal sodium or serosal potassium is reduced, and inhibition under the former condition is prevented by elevating serosal K.

Effect of Ouabain on Bladders Clamped at 100 mV

Perhaps the explanation for the effect of high potassium concentration was related to the known K-ouabain interaction; for instance, the effect of ouabain on red blood cells is inhibited by elevation of the potassium concentration in the medium (Hoffman, 1966). If such interaction were the only explanation,

however, we would expect to find that a decrease in Na_M is associated with a fall in the potassium content of the transport pool, thus accounting for the ouabain effect as cell [K] falls; as previously shown, however, this is not the case (Finn and Nellans, 1972), cell [K] remaining unchanged at low Na_M.

On the other hand, both lowering mucosal sodium and lowering serosal potassium probably bring about a change in the electrochemical potential gradient for sodium across the mucosal barrier of the epithelial cells. In the case of lowering mucosal sodium, there is clearly a decrease in pool size (Finn, 1971), but the decrease is less marked than the decrease in mucosal Na. Since the cell electrical potential in the short-circuited preparation remains unchanged at low mucosal sodium concentrations (Cereijido and Curran, 1965; Reuss and Finn, unpublished data), the result is that the driving force for entry of sodium into the cells becomes less favorable. Similarly, in a low potassium medium, the gradient probably changes in the same direction since the sodium pool rises (Finn and Hutton, 1974). If these assumptions are correct, then one might expect that altering the driving force by voltage clamping in normal Ringer solution might also affect the entry step in the presence of ouabain.

Thus, the experiments shown in Table V were performed. In each case a washout was performed on a bladder whose transepithelial potential was clamped at 100 mV, serosa positive, while mounted in normal Ringer solution. Subsequently the tissue was again loaded with tracer, ouabain was added to the serosal medium (final concentration 5×10^{-4} M), and another wash was performed 90 min later. Again, during this entire time, the bladder PD was clamped at $+100$ mV.

TABLE V

EFFECT OF OUABAIN ON SODIUM KINETICS IN BLADDERS CLAMPED AT $+100$ mV

	J_{1M}	J_{M1}	A_1	J_{S1}	J_{1S}	k_{M1}	k_{S1}
(A) Control (3)	6.34	5.63	17.37	0.796	0.086	0.309	0.048
	±2.25	±2.16	±4.83	±0.180	±0.143	±0.032	±0.007
(B) Clamp, +100 mV (8)	6.30					0.366	0.020
	±1.22					±0.028	±0.008
(C) Clamp, +100 mV + ouabain (8)	3.48					0.337	0.003
	±0.69					±0.043	±0.001
A-B (3)	0.19					−0.046	−0.035
	±3.7					±0.071	±0.002
P difference	NS					NS	<0.01
B-C (8)	2.81					0.028	0.016
	±0.65					±0.027	±0.004
P difference	<0.01					NS	<0.01

Eight bladders were studied. In three of them, washouts were performed in the short-circuited state (A), while clamped at 100 mV, serosa positive (B), and again under 100-mV clamp 90 min after the addition of ouabain (C). In the other five bladders, only two washouts were done under conditions (B) and (C). This is also indicated by the numbers in parentheses.

As shown in Table V, ouabain produced a highly significant decrease in the influx into the transport pool, as well as the expected decrease in the pump rate coefficient; although there is considerable scatter in the controls, these effects were observed in every preparation. In these studies, we do not have an electrical measurement of the net sodium flux (the bladder is not short-circuited); under these conditions, the only determinations which can be made, as stated above, are k_{M1}, k_{S1}, and J_{1M}.

Two further points should be made. First, in the bladders clamped at 100 mV in the absence of ouabain, the influx J_{1M} is not significantly different from control values (short-circuited) (nor is it different from those reported in Table I and previously) (Finn and Rockoff, 1971; Finn and Hutton, 1974). Second, the serosal efflux rate coefficient is significantly lower when the PD is clamped at 100 mV than in control bladders. These points are shown in the three experiments in Table V in which three washouts were performed, including a control under short-circuited conditions.

DISCUSSION

We have shown that ouabain acts at at least two separate sites on transepithelial sodium transport in the toad urinary bladder. First, there is approximately a 70% decrease in the efflux from the cells to the serosal medium. These experiments constitute the first direct evidence of inhibition of the flux through the pump in any epithelium.

Secondly, ouabain effects a decrease in mucosal sodium entry; this decrease becomes manifest when the driving force for entry of this ion becomes less favorable. This was shown in four different types of experiments. Ouabain inhibited sodium entry when the gradient was changed by lowering the mucosal sodium concentration (a phenomenon also observed in frog skin by Biber [1971]), lowering the serosal potassium concentration, and clamping the transepithelial PD at +100 mV. In addition, the effect of lowering mucosal sodium was prevented by raising serosal potassium.

One model which would explain all the data would be to assume that there are two separate modes of entry into the transport pool, only one of which is ouabain inhibitable. Thus, although lowering mucosal sodium might be expected to decrease the rate of sodium entry by either an active or a passive mechanism (Table II), neither changing serosal K (Finn and Hutton, 1974) nor clamping the transepithelial PD at 100 mV (Table V) affected sodium entry in the absence of ouabain as compared to control studies.[2] Yet all three maneuvers unmasked a ouabain-inhibitable step. It

[2] This result differs from that reported in frog skin by Biber and Sanders (1973), who showed a decrease in influx of 20% when skins were open circuited (mean PD = 47 mV) as opposed to short circuited. This is most likely an expression of the apparently more permeable outer membrane in toad bladder than in frog skin (mean J_{1M}/J_{net} in toad bladder is 22.0 [Finn and Rockoff, 1971] and in frog skin is 3.4 [Biber, 1971]).

would appear that when the driving force for sodium entry is made less favorable, less of the entry is via the passive, or ouabain-independent pathway, and proportionately more enters via a perhaps active, or ouabain-inhibitable, pathway. The means by which such a mechanism operates is at present unknown.

These results offer an explanation of the failure of ouabain to affect the measured transport pool, in Ringer solution, since a decrease in both mucosal influx and serosal efflux might lead to maintenance of the pool. Furthermore, since the mucosal fluxes are an order of magnitude greater than those at the serosal side, small (and unmeasurable by our method) changes in the former could keep the pool size constant in the face of even a 75% decrease in serosal efflux. It must be pointed out that the presence of ouabain does not mean that all active transport is necessarily absent and that therefore pool sodium must rise to reach equilibrium with the external sodium.

In previous studies of toad urinary bladder, the addition of ouabain has been shown to produce a rise in total tissue and calculated intracellular sodium (Finn et al. 1966; Herrera, 1968). However, it may well be that most or all of this sodium was contained in nonepithelial elements and, in any event, there is no evidence that any of the rise was in a transport pool. Recently, however, Crabbé (1974) has shown that ouabain lengthens the half time of tracer washout from preloaded bladders without apparently affecting the size of the tissue compartment, although once again there is little evidence that this is the transport compartment.

On the other hand, there is now abundant evidence that the pool we are measuring is the transport pool, and that it is contained between two functionally identifiable barriers (Finn, 1971; Finn and Rockoff, 1971; Finn and Nellans, 1972; Finn and Hutton, 1974). A problem in the interpretation of epithelial transport mechanisms has arisen because of the use of different methods of determining this pool. For instance, most previous kinetic approaches have been inadequate because the measured pool probably represented an already transported compartment (Zerahn, 1969). Furthermore, methods which depend on the difference between effects on electrical parameters or fluxes of ouabain and amiloride (Cuthbert, 1971; Cereijido et al., 1974) are obviously grossly inadequate since these determinations require the assumptions that ouabain affect only the serosal border and amiloride only the mucosal. Both assumptions are false: ouabain inhibits entry, as shown here and by Biber (1971), as well as exit, and although amiloride clearly inhibits entry, there are no data which support the contention that this drug does not inhibit the pump.

In conclusion, ouabain inhibits both mucosal entry into, and serosal exit from, the transport pool in toad urinary bladder after addition of this compound to the serosal surface. Since there is at present no evidence that ouabain

enters the cells, it is likely that its immediate site of action is on the serosal surface. It would be of great interest to determine whether the action at the mucosal border is mediated in the same way as that at the serosal border, or whether indeed the two opposing (and functionally quite different) barriers are independent of one another. Although we cannot be certain of this, recent evidence from this laboratory (Reuss and Finn, 1975) indicates that a decrease in the serosal transmembrane potential follows that of the mucosal trans-membrane potential within less than 25 ms after the addition of amiloride to, or the removal of sodium from, the mucosal surface. These data lead us to believe that the mucosal and serosal borders may well "signal" one another in some way, and that the action of ouabain and of vasopressin (Morel and Bastide, 1965; Janacek and Rybova, 1970; Finn, 1971) at two sites is not surprising.

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