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# INDUCTION OF REVERSE FLOW OF $Na^+$ THROUGH THE ACTIVE TRANSPORT PATHWAY IN TOAD URINARY BLADDER

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## Summary

When active $Na^+$ transport across the toad urinary bladder was abolished by ouabain, a 'reversed' short circuit current could be induced by an $Na^+$ concentration gradient. This reversed current was increased by vasopressin and inhibited by amiloride and appears to represent net $Na^+$ movement 'backwards' through epithelial cells which normally participate in active $Na^+$ transport across the bladder.

Active sodium transport across an epithelial cell layer may be regarded as the net effect of two oppositely directed, unidirectional rate processes. Ussing [1] proposed that the ratio of the unidirectional fluxes in the active sodium transport pathway provided a measure of the effective driving force of the sodium pump in the isolated frog skin, $E_{Na^+}$. More recently, however, it has been widely recognized that the transmural flux-ratio may not be uniquely related to the active transport process because a significant portion of undirectional fluxes measured in isolated epithelial preparations may traverse extracellular pathways; either between cells [2,3] (paracellular shunts) or through the 'damaged edge' of the isolated epithelium [4]. Quantitative measures of shunt permeability in several $Na^+$-transporting epithelia have, in fact, suggested that virtually all of the $Na^+$ 'backflux' across isolated rabbit ileum [5,6], rabbit colon [7], toad bladder [8] and turtle colon [9] traverses the paracellular route, i.e., that a backflux through cellular pathways involved in active transport across these tissues is not detectable. This result is not unexpected, however, since the apparent e.m.f. of the active path in the toad bladder [10] and turtle colon [9] appears to be of the order of 120 mV. The predicted 'backflux' through the cellular active trans-

port path would thus be on the order of $10^{-2}$ $\mu$equiv/cm$^2$ per h and perhaps difficult to resolve in a typical unidirectional flux measurement [9].

Recent studies on the relation between active $Na^+$ transport and metab olism in the toad bladder have suggested that $Na^+$ entry into the active trans port pool from the serosal side is undetectable. Such experiments indicate that there is no 'recycling' of serosal $Na^+$ across the basolateral membranes, i.e., the coupling between active $Na^+$ flow and $CO_2$ production is independent of serosal $Na^+$ concentration [11]. These experiments, coupled with the tissue labeling studies of MacKnight et al [12], suggest that serosal $Na^+$ may not have access to the '$Na^+$-transport pool'.

Recent studies of the relation between active $Na^+$ transport and metab-may be possible to measure an isotopic $Na^+$ backflux through the cellular path involved in active transport. O'Neil and Helman [13] reported that in non edge-damaged frog skin, under conditions of zero electrochemical potential gradient, it was possible to detect an isotopic $Na^+$ backflux which was increased by vasopressin and inhibited by amiloride. Similarly, Biber and Mullen [14,15] reported that in non edge-damaged frog skins under conditions of zero electrochemical potential, the unidirectional $Na^+$ back-flux exhibited saturation kinetics, was enhanced by ouabain and, at least at low serosal $Na^+$ concentrations, was inhibited by amiloride. These studies, however, because they were conducted under conditions of zero electrochemical potential gradient, provide no information as to whether, under optimum conditions, it may be possible to induce a net flow of $Na^+$ backwards, through the cellular path responsible for active transport.

Thermodynamic considerations suggest that the apparent permeabil-ity of an active transport pathway may depend on 'energetic' as well as 'passive' properties [16]. Despite these complexities it seems reasonable to expect that agents which inhibit metabolically-linked $Na^+$ transport (e.g. ouabain) might decrease the 'barrier' to backflow of $Na^+$ through cells involved in active transport. In this circumstance an appropriate external driving force would be expected to produce a net backflow of sodium through $Na^+$-transporting cells. We report here that in the presence of ouabain and an $Na^+$ concentration gradient net flow of $Na^+$ was induced from the serosal to the mucosal side of the toad bladder through a pathway which, on the basis of responses to vasopressin and amiloride, appears to represent cells which are normally engaged in active $Na^+$ transport across the tissue.

We used urinary bladders from Dominican toads since it is well-known that under normal circumstances $Na^+$ is the only ion actively transported by this tissue [17,20]. Isolated hemibladders were mounted in Ussing chambers (area, 8 cm$^2$) equipped with four 3 M KCl electrodes, two for measuring the transepithelial electrical potential difference (PD) and two for passing current across the tissue. A driving force favorable to the back-wards movement of $Na^+$ was established by bathing the mucosal side of the tissue with a sodium-free, choline-Ringer's solution, while the serosal side was bathed with Ringer's solution containing 112 mM $Na^+$. The transmural PD was voltage clamped at zero mV and the short circuit current ($I_{sc}$) was monitored continuously.

Fig. 1 shows a representative experiment. After washing the mucosal side of the bladder several times with $Na^+$-free Ringer's solution $I_{sc}$ is reduced to a low value. Although the $Na^+$ concentration in this solution was not routinely measured in these experiments, previous studies have indicated that several washes of the mucosal side with $Na^+$-free Ringer's solution reduces the $Na^+$ concentration to values less than 1 mM. Note, however, that despite the counter e.m.f. due to the $Na^+$ gradient, the direction of $I_{sc}$ is nevertheless consistent with active $Na^+$ transport from mucosa to serosa. At the time indicated the serosal solution was exchanged for one containing 10 mM ouabain. $I_{sc}$ declined to zero and then reversed in sign, such that the direction of net positive charge transfer was from serosa to mucosa, opposite to that of the normal active $Na^+$ current. The reversal of current flow was produced by ouabain only in the presence of an $Na^+$ gradient from serosa to mucosa. Addition of 10 m units of vasopressin to the serosal side increased the reversed current from $-3.0$ to $-8.0$ $\mu$A. Mucosal addition of 0.1 mM amiloride abolished the reversed current virtually instantaneously. Table I shows the pooled results from a series of such experiments in which the effects of vasopressin and amiloride on the short-circuit current were studied. Vasopressin significantly increased the reversed current by 5.5 $\mu$A. Amiloride reduced the reversed current by 8.4 $\mu$A to a value not different from zero. The mean total tissue conductance ($m\Omega^{-1}/8$ cm$^2$) was 1.76 ± 0.07 just prior to the addition of vasopressin. After the addition of vasopressin the conductance increased to 2.11 ± 0.14, while after the addition of amiloride the conductance decreased to 1.64 ± 0.07.

These experiments indicate that it is possible to establish a 'reversed' $I_{sc}$ across the isolated toad bladder by applying a counter e.m.f. in the form of an $Na^+$ gradient from serosa to mucosa and inhibiting active $Na^+$ transport with ouabain. The reversed $I_{sc}$ is stimulated by vasopressin and inhibited by amiloride, i.e., in a qualitative sense the reversed current responds to these

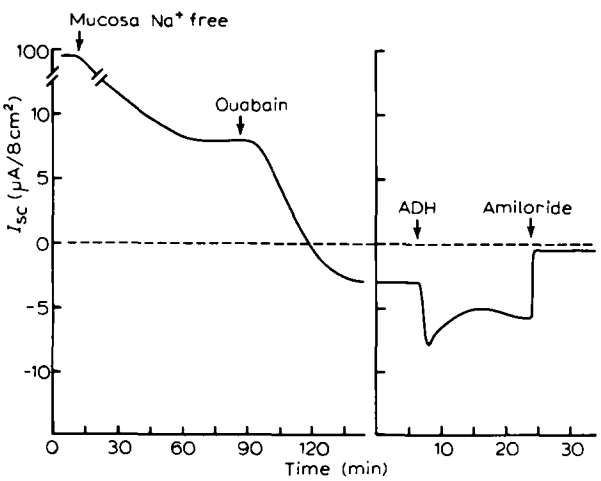


**Fig. 1. Short circuit current ($I_{sc}$) across the toad bladder as a function of time. Positive values indicate current flow from mucosal side to serosal side. The time axis is interrupted to emphasize the change in time scales.**

agents in a manner which is identical to the $I_{sc}$ of the normal, actively transporting bladder. There is good evidence that vasopressin and amiloride affect active $Na^+$ transport across epithelial cell layers by changing the $Na^+$ permeability of the apical cell membrane [18,19]. In the present experiments the effect of these agents is clearly on permeability since it is observed in the absence of active transport. The effects of vasopressin and amiloride on epithelial conductance are also consistent with a primary effect on $Na^+$ permeability. The reversed currents observed in these experiments appear to represent, at least in part, the passive movement of $Na^+$ down an electrochemical potential gradient through the active $Na^+$-transport path, broadly defined as the cells which are normally responsible for active $Na^+$ transport across the bladder.

It should be noted that the identification of the small reversed $I_{sc}$ measured in this study (0.5 $\mu A/cm^2$) with a net ionic flux may pose some technical difficulties. A net flux of this magnitude might be obscured, for instance, by the noise inherent in tracer flux measurements. In addition, although bladders of Dominican toads possess less carbonic anhydrase-containing cells than those from toads of Colombian origin [20], the bladders of Dominican toads are not devoid of the enzyme. It might be argued that these bladders could exhibit an 'acidification current' consistent with the magnitude of the reversed $I_{sc}$ observed in these studies, but below the limit of detectability of the pH-stat titration technique [20]. In the present experiments, however, the ouabain-induced reversal of $I_{sc}$ was only observed in the presence of an $Na^+$ gradient favoring serosa to mucosa $Na^+$ movement. In addition, the reversed $I_{sc}$ was altered by vasopressin and amiloride in a manner consistent with effects of these agents on normal, active $Na^+$ transport. These observations strongly suggest that the reversed $I_{sc}$ is the result of $Na^+$ movement through the same cellular path which is the normal route of active $Na^+$ absorption.

The induction of a reverse flow of $Na^+$ through the active transport path in the toad urinary bladder provides evidence that under appropriate conditions serosal $Na^+$ can enter cells normally responsible for active $Na^+$ absorp-

TABLE I

EFFECT OF OUABAIN, VASOPRESSIN AND AMILORIDE ON THE SHORT CIRCUIT CURRENT IN THE PRESENCE OF A SODIUM GRADIENT

$I_{sc}$ ($\mu A/8\ cm^2$)

| | $Na^+$-Ringer's solution | Ouabain ($10^{-3}$ M) | Vasopressin (0.1 units/ml) | Amiloride ($10^{-4}$ M) |
|---|---|---|---|---|
| | 100 | −8.6 | −13.0 | + 3.0 |
| | 115 | −3.0 | − 6.5 | −0.6 |
| | 40 | + 3.1 | − 5.0 | + 1.3 |
| | 49 | −5.1 | −10.5 | −2.8 |
| | 105 | −5.0 | −12.0 | −4.6 |
| | | −7.0 | −12.0 | −5.0 |
| $\bar{x}$ | 81.8 | −4.3 | − 9.8 | −1.4 |
| S.E. | ±15.3 | ± 1.7 | ± 1.3 | ± 1.3 |

tion. The mechanism by which $Na^+$ crosses the basolateral membranes of these cells has not been discerned, but at least two possibilities may be envisioned. $Na^+$ might simply diffuse across the basolateral membrane or $Na^+$ might actually traverse the ouabain-sensitive $Na^+$ pump in reverse. Ouabain might be expected to facilitate $Na^+$ backflux in the former case by reducing active $Na^+$ extrusion and, in the latter, perhaps by decreasing the energetic barrier to backflow through the pump. Chen and Walser [21] reported, however, that in a sac preparation of the toad bladder bathed on both sides by $Na^+$-Ringer's solution and voltage-clamped at 150 mV, serosal side positive; the serosal to mucosal, unidirectional $Na^+$ flux was decreased in the presence of ouabain.

In a recent study Wolff and Essig [22] obtained evidence for a net flow of $Na^+$ from serosa to mucosa through $Na^+$-transporting cells of the toad bladder bathed on both sides by $Na^+$-Ringer's solution and voltage-clamped at a serosal-positive PD which exceeded the apparent e.m.f. of the active path.

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## References

1 Ussing, H.H. and Zerahn, K. (1951) Acta Physiol. Scand. 23, 110—127
2 Ussing, H.H. and Windhager, E.E. (1964) Acta Physiol. Scand. 61, 484—504
3 Fromter, E. and Diamond, J.M., (1972) Nat. New Biol. 235, 9—13
4 Helman, S.I. and Miller, D.A. (1973) Am. J. Physiol. 225, 972—977
5 Frizzell, R.A. and Schultz, S.G. (1972) J. Gen. Physiol. 59, 318—346
6 Nellans, H.N., Frizzell, R.A. and Schultz, S.B. (1974) Am. J. Physiol. 226, 1131—1141
7 Frizzell, R.A., Koch, M.J. and Schultz, S.G. (1976) J. Membrane Biol. 27, 297—316
8 Saito, T., Lief, P.D. and Essig, A. (1974) Am. J. Physiol. 226, 1265—1271
9 Dawson, D.C. (1978) J. Membrane Biol., 37, 213—233
10 Yonath, J. and Civan, M.M. (1971) J. Membrane Biol. 5, 366—385
11 Beauwens, R. and Al-Awqati, Q. (1976) Am. J. Physiol. 231, 222—227
12 MacKnight, A.D.C., Civan, M.M. and Leaf, A. (1975) J. Membrane Biol. 20, 365—386
13 O'Neil, R.G. and Helman, S.I. (1976) Am. J. Physiol. 231, 164—173
14 Biber, T.U.L. and Mullen, T.L. (1976) Am. J. Physiol. 231, 995—1001
15 Biber, T.U.L. and Mullen, T.L. (1977) Am. J. Physiol. 232, C67—C75
16 Essig, A. and Caplan, S.R. (1968) Biophys. J. 8, 1434—1457
17 Leaf, A. (1965) Ergeb. Physiol. 56, 216—263
18 Civan, M.M. and Frazier, H.S. (1968) J. Gen. Physiol. 51, 589—605
19 MacKnight, A.D.C., Leaf, A. and Civan, M.M. (1971) J. Membrane Biol. 6, 127—137
20 Rosen, S., Oliver, J.A. and Steinmetz, P.R. (1974) J. Membrane Biol. 15, 193—205
21 Chen, J.S. and Walser, M. (1975) J. Membrane Biol. 21, 87—98
22 Wolff, D. and Essig, A. (1977) Biochim. Biophys. Acta 468, 271—283