IDENTIFICATION OF A BASOLATERAL Na/H ANTIPORTER IN URINARY BLADDER OF FLOUNDER, PSEUDOPLEURONECTES AMERICANUS

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Na/H antiporters have been identified in the apical and basolateral membrane of epithelial cells. The apical isoform is one component of an electrically silent active Na^+ absorption pathway, but the function of the basolateral isoform of the exchanger is less well understood. We recently showed that the basolateral membrane of turtle colon, an electrogenic Na^+ absorptive epithelium, expresses a high level of amiloride-sensitive Na/H exchange activity (Post & Dawson, FASEB J. 4:A549, 1990). The purpose of these experiments was to determine if Na/H exchange activity could be identified in the basolateral membrane of the flounder urinary bladder, an electrogenic K^+ secretory epithelium. We assayed for the presence of exchange activity by measuring ouabain-insensitive, amiloride-inhibitable transmural fluxes of $^{22}Na^+$ across sheets of bladder which had been apically permeabilized with the pore-forming antibiotic amphotericin-B, and by imposing large transmural gradients of Na^+ or H^+ to induce counterflow or "transacceleration" of the $^{22}Na^+$ flow.

Flounder urinary bladder was initially mounted in Ussing chambers (0.287 cm^2) as previously described (Wilkinson & Dawson, Bull. M.D.I.B.L. 29:108-109, 1990). The Ringer's contained (in mM): 140 Na^+, 147.5 Cl^-, 2.5 K^+, 1.5 Ca^{2+}, 1.0 Mg^{2+}, 15 HEPES, and 10 glucose, at a pH of 7.5. 100 µM verapamil was present in the serosal bath to minimize smooth muscle activity and 100 µM ouabain was present to inhibit basolateral Na/K-ATPase activity. Unidirectional $^{22}Na^+$ fluxes were determined in similar solutions except that chloride was replaced by gluconate, and in some cases K^+ replaced all but 2 mM of the Na^+. All Ringer's solutions used for flux determinations were initially adjusted to a pH of 6.5 to ensure maximal activation of the antiporter. Transmural fluxes of $^{22}Na^+$ were measured according to the "sample and replace" paradigm of Dawson (J. Membr. Biol. 37:213-233, 1977), except that 5 µCi $^{22}Na^+$ was used, the interval between samples was shortened to 10 minutes, and the sample volume (1 ml) was 25% of the bath volume (4 ml).

Table 1 presents evidence for basolateral Na/Na exchange in flounder urinary bladder. In the presence of serosal ouabain and a 140 to 2 mM mucosal (M) to serosal (S) Na^+ gradient the unidirectional rate coefficient for S to M $^{22}Na^+$ flow (λ^*_{SM}) was greater than the unidirectional rate coefficient for M to S $^{22}Na^+$ flow (λ^*_{MS}). This result may reflect the presence of apical Na/Na exchange, mediated by the NaCl cotransporter (Stokes, J. Clin. Inv. 74:7-16, 1984), in series with basolateral Na/Na exchange, mediated by a Na/H exchanger. Permeabilizing the apical membrane (10 µM amphotericin B) revealed a large transacceleration of λ^*_{SM} by the M to S Na^+ gradient. The addition of amiloride (0.5 mM) to the serosal bath reduced both λ^*_{SM} and λ^*_{MS} to near zero. The magnitude of the Na/Na exchange activity can be best appreciated by noting that the amiloride-sensitive portion of λ^*_{SM} was 50 times greater than the amiloride-sensitive portion of λ^*_{MS} (i.e $\Delta SM/\Delta MS=50$). These results are consistent with the notion that a high level of amiloride-sensitive Na/Na exchange activity is present in the basolateral membrane of flounder urinary bladder. Because these are well known characteristics of the Na/H exchanger (Aronson, Ann. Rev. Physiol. 47:545-60, 1985), we assayed for Na/H exchange by using a proton gradient to drive $^{22}Na^+$ counterflow.

Figure 1 depicts λ^*_{SM} first in the absence and then in the presence of an M to S proton gradient. Both baths contained K gluconate Ringer's solution at pH 6.5; the serosal bath also contained ouabain (100 µM) and verapamil (100 µM). Initially the opposing unidirectional rate coefficients were low and symmetric (λ^*_{MS} not shown), as expected for ouabain treated tissue in the absence of transmural ion gradients.

Permeabilization of the apical membrane by amphotericin (10 μM) in the absence of a Na^+ or H^+ gradient revealed that a basolateral route for $^{22}Na^+$ flow was present (indicated by the increase in λ^*_{SM}. Imposition of an M to S H^+ gradient, by alkalinizing the serosal bath to pH 8.5 with KOH, transaccelerated λ^*_{SM}, as expected for obligatory Na/H exchange. The addition of 1 mM amiloride to the serosal bath reduced λ^*_{SM} to below the level measured prior to apical permeabilization, consistent with complete block of the basolateral pathway for Na^+ movement.

The results presented in this report support the notion that robust Na/H exchange activity is expressed in the basolateral membrane of winter flounder urinary bladder. As in other tissues, the flounder bladder Na/H exchanger was able to catalyze both Na/Na and Na/H exchange, and was inhibited by amiloride. It will be of interest to determine if the antiporter plays a role in the regulation of transepithelial transport as suggested by Harvey and Ehrenfeld (J. Gen Physiol. 92:793-810, 1988).

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Table 1
Unidirectional Rate Coefficients for $^{22}Na^+$ Flow Across Flounder Urinary Bladder (Mean ± SD)

	λ^*_{MS} (cm/h x 10^3)	λ^*_{SM} (cm/h x 10^3)
Serosal ouabain (100 μM)	1.2 (0.2)	19.9 (3.3)
Mucosal amphotericin-B (10μM)	7.0 (0.8)	185.9 (9.6)
Serosal amiloride (0.5 mM)	3.4 (0.7)	6.9 (2.3)
Δ	2.6	179

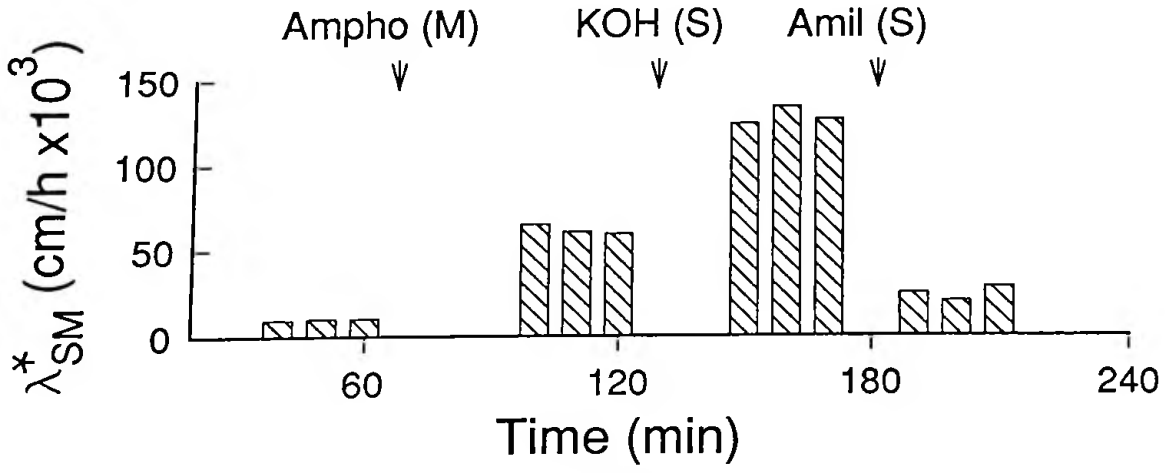

Figure 1 Flounder urinary bladder possesses basolateral Na/H antiporters. KOH addition to the serosal bath imposed an M to S H^+ gradient (mucosal pH = 6.5; serosal pH = 8.5) and transaccelerated the rate coefficient for S to M $^{22}Na^+$ flow (λ^*_{SM}) across amphotericin-B (ampho) permeabilized tissue. Subsequent serosal addition of amiloride (1 mM) reduced λ^*_{SM}.