ANION CURRENTS ACROSS BASOLATERAL MEMBRANES OF URINARY BLADDER OF WINTER FLOUNDER (Psuedopleuronectes americanus)

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The isolated urinary bladder of the Winter Flounder actively secretes potassium and this net potassium flow can be measured directly as a short circuit current (Isc). Previous studies (Bull. M.D.I.B.L. 20:84,1982) showed that the apical membrane is highly selective for potassium. Thus, under short circuit conditions all of the Isc across the apical membrane is due to net potassium exit from the cell. At the basolateral membrane this current (from serosal bath to cell) must be due to conductive anion exit and/or conductive cation entry.

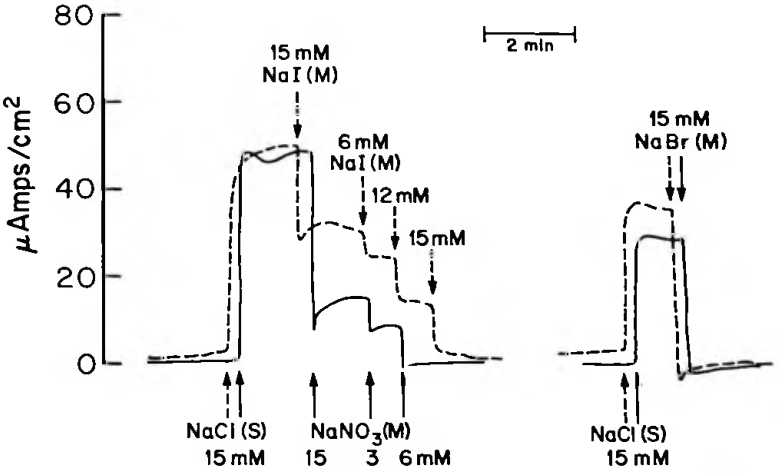
In a previous report (Bull. M.D.I.B.L. 23:26,1983) we presented evidence for the existence of a substantial chloride conductance in the basolateral membranes of flounder urinary bladder epithelial cells. It was possible to measure basolateral chloride currents in isolated portions of bladder which had been treated with amphotericin-B in order to increase the chloride conductance of the apical membrane. The aim of the present experiments was to further characterize the basolateral anion currents, particularly with regard to the anion selectivity of the basolateral membrane.

Portions of flounder urinary bladder were mounted as flat sheets in Ussing chambers as previously described (Bull. M.D.I.B.L. 19:46,1981), and were treated with ouabain (0.1 mM, serosal) to abolish the active transport on both sides by Cl-free Ringer's solutions which contained (in mM) Na: 147.5, K: 2.5, Ca: 1.5, Mg: 1.0, gluconate: 147.5, and Hepes: 15.0. The mucosal and serosal solutions were stirred with air and the pH was approximately 7.5. The serosal solution also contained Verapamil (10 μM) to inhibit smooth muscle contractions.

Anion gradients were imposed across the tissue by adding a small volume of the Na salt of the anion to the mucosal or serosal bath. Anion currents were measured as the Isc induced by the transmural anion emf in the presence of mucosal amphotericin-B. As indicated previously (Bull. M.D.I.B.L. 23:26,1983) this method exploits the fact that the so-called "one-sided" polyene channel (Marty and Finkelstein, J.G.P. 65:515,1975) is cation selective, but nevertheless exhibits a significant anion conductance. The data of Marty and Finkelstein are consistent with a ratio for P_{Na}/P_{Cl} of about 7.3.

We tested a series of anions (Cl^-, Br^-, NO_3^-, SCN^-, I^-, and acetate) for their ability to produce anion currents when gradients were imposed in the presence of Cl-free solutions. The criteria for passive ion flows was (1) the prompt development of positive current from serosa to mucosa after mucosal addition, (2) reduction of this current to zero after abolishing the applied emf by adding an identical anion concentration to the serosal bath, and (3) the development of a positive current mucosa to serosa following a further increase in the serosal ion concentration. Of the anions tested all but acetate produced passive basolateral currents in the presence of amphotericin-B.

We attempted to estimate the anion selectivity of the amphotericin treated bladder by initiating a current by adding an anion, say Cl^-, to the mucosal (or serosal) side and then determining the relative ability of other anions to nullify this current when added to the opposite side. Figure 1 shows representative experiments in which Br^-, NO_3^-, and I^- were compared by determining the mucosal concentration of each anion necessary to nullify a current induced by the addition of 15 mM Cl to the serosal bath. The figure shows that 15 mM Br^- reduced Isc to zero whereas 24 mM NO_3^- and 48 mM I^- were required to nullify the Isc. Similar experiments (not shown) showed that equimolar quantities of SCN would nullify a Cl^- current. This result suggested a selectivity sequence of $Cl^- \sim Br^- \sim SCN^- > NO_3^- > I^-$.



We also investigated the anion vs cation selectivity of the polyene-treated bladder by comparing the currents generated by various cation salts of permeable anions and by measuring currents induced by gradients of the Na and K salts of impermeable anions such as gluconate or sulfate. These experiments were prompted by previous observations (Bull. M.D.I.B.L. 21:29,1981) which suggested that in some bladders a basolateral K conductance was detectable. In several bladders the Na, K and choline salts of Cl were compared and generated virtually identical currents. In addition a current induced by the addition of 15 mM KCl to the mucosal bath was abolished by the addition of 15 mM choline-Cl to the serosal bath. Gradients of K gluconate (in Na gluconate Ringer's) or Na gluconate (in K gluconate Ringer's) produced no detectable Isc.

The interpretation of the magnitudes of transmural anion currents across polyene-treated bladders is complicated by the fact that measured currents reflect the properties of at least two membrane in series, the apical and basolateral membranes of the epithelial cell layer. Given the relatively low permeability of the polyene channels for anions it is likely that the apical membrane was a significant barrier to steady-state anion flow or was even the rate limiting resistance. The ambiguity concerning the absolute rates, however, does not compromise the two major conclusions of this study: (1) The basolateral membrane must be highly selective for anions and (2) a variety of anions can permeate basolateral anion channels.