INORGANIC MERCURY INHIBITS NaCl COTRANSPORT IN FLOUNDER (PSEUDOPLEURONECTES AMERICANUS) URINARY BLADDER

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In a previous report (Wilkinson and Dawson, Bull. M.D.I.B.L. 29:108-109; 1990) we presented evidence that inorganic mercury inhibited K^+ secretion by the flounder urinary bladder via an indirect mechanism of action: blockade of apical thiazide-sensitive NaCl cotransport. Here we present the results of transmural flux measurements designed to test directly for an effect of divalent mercury on NaCl absorption. The results suggest that Hg^{2+} may be a relatively selective and reversible inhibitor of the apical NaCl cotransporter.

Sheets of flounder urinary bladder were mounted in Ussing chambers as previously described (Wilkinson & Dawson, Bull. M.D.I.B.L. 29:108-109, 1990), and bathed by a Ringer's solution which 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 serosal verapamil was also present to inhibit smooth muscle activity. The tissue was short circuited (serosal bath as reference) and the current (I_{SC}) and conductance (G_T) were continuously monitored. The I_{SC} in this tissue results directly from K^+ secretion (Dawson & Frizzell, Pflügers Arch 414:393-400, 1989). The mucosal to serosal rate coefficient for $^{22}Na^+$ flow (λ^*_{MS}) was determined as described previously (Dawson & Andrew Bull M.D.I.B.L. 19:46-49, 1979), except that the flux periods were shortened to 15 min. The rate coefficient was calculated as: $\lambda^*_{MS} = J^*_{MS}/C^*_M$, where J^*_{MS} is the mucosal to serosal flux of tracer and C^*_M is the concentration of tracer in the "hotside" bath (in cpm/ml). λ^*_{SM} was determined in a similar fashion. λ^*_{MS} is a direct measure of Na^+ absorption via the hydrochlorothiazide (HCT) sensitive NaCl cotransporter (Stokes, J. Clin. Invest. 74:7-16, 1984)

Figure 1 is a typical experiment (n=8) showing the effect of Hg^{2+} on Na^+ absorption and K^+ secretion in a single tissue. Each bar represents a 15 minute flux period. The addition of 1.5 µM $HgCl_2$ to the mucosal bath produced about a 50% inhibition of Na^+ absorption and a profound inhibition of I_{SC}. This inhibition was at least partially reversed by the mucosal addition of 1 mM dithiothreitol (DTT) to chelate the Hg^{2+}. Subsequent mucosal addition of 100 µM HCT, a specific inhibitor of the NaCl cotransporter (Stokes, J. Clin. Invest. 74:7-16, 1984) markedly attenuated the absorptive Na^+ flux and the I_{SC}. The S to M fluxes of $^{22}Na^+$ (not shown) were unaffected by these maneuvers.

These results are consistent with the hypothesis that mercury reversibly inhibits apical NaCl cotransport in flounder urinary bladder. In separate experiments we characterized the selectivity and reversibility of Hg^{2+} block by monitoring the change in I_{SC} in perfused tissues, as previously described (Wilkinson & Dawson, Bull. M.D.I.B.L. 29:108-109, 1990). Inhibition of I_{SC} by 1 µM Hg^{2+} was completely reversible within 30 min of exposure, but after 90 min of exposure to Hg^{2+} recovery was only about 50% reversible. Other divalent cations such as Ni^{2+} (5 µM), Cu^{2+} (3 µM), Co^{2+} (4 µM), or Cd^{2+} (2 µM) were without effect. In addition, the organic mercurial p-chloromercuribenzenesulfonic acid (PCMBS) had no effect at a concentration of 50 µM, although a slight inhibition of I_{SC} was noted at 150 µM.

These and previous observations can be organized into a speculative model for the action of Hg^{2+} on the flounder urinary bladder. The efficacy of Hg^{2+} and the lack of effect of PCMBS at inhibiting transport suggests that the divalent form of mercury is the active agent. The complete and rapid reversal of the effects of Hg^{2+} either by chelation (fig 1) or by washout in perfusion chambers (not shown) suggests an extracellular site of action. Divalent mercury could act by binding to a site on the HCT sensitive

cotransporter (for example, the Na^+ binding site) and thus inhibit the translocation of NaCl. The consequent reduction in Na^+ entry indirectly attenuates apical K^+ exit, perhaps by means of a hyperpolarization of the apical membrane potential as suggested by the results of Duffy and Frizzell (Fed. Proc., 43:444, 1984).

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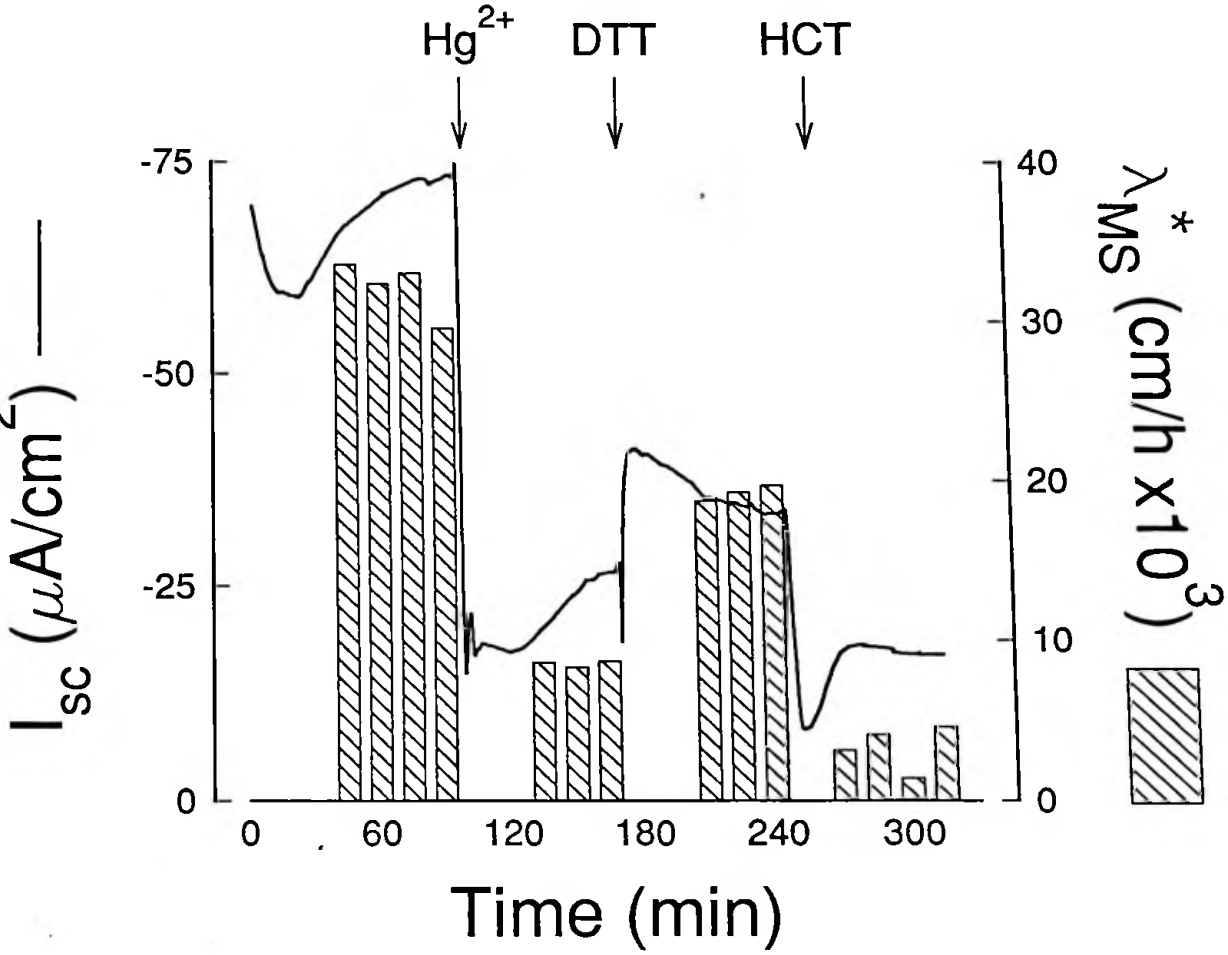

Figure 1 Mucosal addition of 1.5 μM Hg^{2+} inhibited and 1 mM dithiothreitol (DTT) partially restored Na^+ absorption (right hand axis; unidirectional rate coefficient for M to S $^{22}Na^+$ movement, λ^*_{MS}) and K^+ secretion (left hand axis) by the flounder urinary bladder.