Hg^{++} INHIBITS K^+ SECRETION BUT DOESN'T BLOCK APICAL K CHANNELS IN URINARY BLADDER OF WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS): POSSIBLE INHIBITION OF NaCl ENTRY

Daniel J. Wilkinson and David C. Dawson
Department of Physiology, University of Michigan Medical School,
Ann Arbor, MI 48109.

The urinary bladder of the winter flounder actively secretes K^+, and the rate of K^+ secretion can be measured as a short-circuit current when the tissue is mounted as a flat sheet in an Ussing chamber (Dawson and Frizzell, Pfluegers Arch. 414:393-400, 1989). Blockade of the K^+ secretory current by mucosal barium as well as analysis of fluctuations in the transmural K^+ current (Van Driessche et al., Bull. M.D.I.B.L. 25:1-3, 1985) provide evidence for a population of apical K channels which mediate K^+ exit from the cells to the luminal solution. Previous studies (Chang et al., Bull. M.D.I.B.L. 25:44-45, 1985; Venglarik and Dawson, Bull. M.D.I.B.L. 26:1-4, 1986) showed that exposure of the apical membranes to concentrations of $HgCl_2$ from 0.5 to 1 μM produced a reversible inhibition of the K^+ secretory current. We speculated that the divalent mercury ion (Hg^{++}) might be acting, by analogy with barium ion (Ba^{++}), as a reversible blocker of the apical K channels, and we tested this hypothesis in the experiments reported here. Fluctuation analysis was used to detect possible reversible blockade of apical K channels, and step increases in the mucosal K^+ concentration were used to assay the apparent transepithelial K conductance. The results provided no evidence for Hg^{++} blockade of K channels, but similarities between the effects of mucosal $HgCl_2$ or hydrochlorothiazide (HCT) and Na-free or Cl-free mucosal solutions suggested that Hg^{++} inhibition of K^+ secretion may be secondary to inhibition of NaCl entry.

Urinary bladders were removed from flounder and mounted in a perfusion chamber as described previously (Van Driessche et al, Bull. M.D.I.B.L. 25:1-3, 1985). All bladders were perfused on both sides with a Ringer's solution that contained (in mM) Na^+: 147.5, Cl^-: 147.5, K^+: 2.5, Ca^{++}: 1.5, Mg^{++}: 1.0, HEPES: 15.0, and glucose: 10. The pH of the Ringer's solution was 7.5. The serosal perfusate also contained 20 μM verapamil to inhibit smooth muscle contraction. Short-circuit current (I_{sc}) was monitored continuously, and transepithelial conductance (g_t) was measured periodically using a brief (~1 sec), 10 mV pulse. The apparent transepithelial conductance to K^+ was assessed by measuring the change in I_{sc} induced by raising the mucosal K^+ concentration from 2.5 to 12.5 mM (change in $[K^+]_m$ = 10 mM), using K-Gluconate to raise $[K^+]_m$. Fluctuations in the I_{sc} were analyzed periodically as described by Van Driessche, Chang, and Dawson (Bull. M.D.I.B.L. 25:1-3, 1985). As shown in that study, two Lorentzian components were discernible in power density spectra of the K^+ secretory current. One appeared spontaneously and was thought to represent the spontaneous gating of apical K channels. A second was induced by mucosal Ba^{++} and was considered to reflect the reversible blockade of the apical K channels by the divalent ion.

Mucosal $HgCl_2$ at a concentration of 0.5 μM decreased the K^+ secretory current (I_{sc}) in ten bladders from 10.3 $\pm$ 2.2 to 5.2 $\pm$ .9 $\mu A/cm^2$ (Mean $\pm$ SE, n = 10), a 42% reduction, and increased the transepithelial conductance from 0.49 $\pm$ .11 to 0.58 $\pm$ .12 mS/cm^2, a 22% increase. Fluctuations in the K^+ secretory current were monitored both before and after inhibition by mucosal Hg^{++} in seven of these experiments. The corner frequency of the spontaneous Lorentzian component was not altered by Hg^{++} (23.8 $\pm$ 1.2 Hz before vs. 23.6 $\pm$ 2.0 Hz after), but the low frequency plateau was reduced from 0.306 $\pm$ .092 to 0.116 $\pm$ .021 (units = 10^{-20} A^2 sec/cm^2), a 47% reduction. Addition of mucosal $HgCl_2$ in the presence of 3 mM $BaCl_2$ reduced the low frequency plateau of the Ba^{++}-induced Lorentzian component but did not change the Ba^{++}-inhibited I_{sc}. These results suggested that the inhibition of K^+ secretion by $HgCl_2$ was not due to channel blockade by Hg^{++} or to an indirect decrease in K conductance but rather to some other

effect which decreased the driving force for K^+ exit from the cells.

To investigate possible changes in transepithelial K conductance, a 10 mM step increase in mucosal K^+ concentration was applied, first in the absence and again in the presence of 0.5 μM $HgCl_2$. The change in I_{sc} produced by the increase in mucosal K^+ was completely blocked by 3 mM $BaCl_2$ in the mucosal perfusate, with or without mucosal $HgCl_2$, indicating that the magnitude of the change in I_{sc} was a reflection of the apparent transcellular K conductance. Mucosal $HgCl_2$ increased the K^+-induced change in I_{sc} by more than two fold, from -5.3 $\pm$ 1 to -12.0 $\pm$ 2 $\mu A/cm^2$ (n = 5), indicating a substantial increase in K conductance despite a decrease in K^+ secretion. This result reinforced the notion that the reduction in K^+ exit across the apical membrane was brought about by a decrease in the driving force.

Because it seemed likely that the effects of Hg^{++} were indirect, we investigated the effects of three other experimental maneuvers that indirectly reduce K^+ secretion: removal of mucosal Cl^- (replaced by gluconate), removal of mucosal Na^+ (replaced by N-methyl-D-glucamine), and addition of mucosal hydrochlorothiazide (100 uM). Each of these maneuvers was expected to attenuate apical entry of NaCl (Stokes, J. Clin. Invest. 74:7-16, 1984), and each produced effects that were qualitatively similar to those of mucosal $HgCl_2$: a reduction in K^+ secretion accompanied by an increase in K conductance. These results are consistent with the notion that the reversible inhibition of K^+ secretion by Hg^{++} is not the result of K channel blockade but, instead, reflects the reversible inhibition by Hg^{++} of some other process, perhaps the coupled entry of NaCl into the cells. (This work was supported by grant NIEHS 1-P50-ES-03828-04 to the Center for Membrane Toxicity Studies.)