# BLOCKADE OF APICAL K CHANNELS IN FLOUNDER (PSEUDOPLEURONECTES AMERICANUS) URINARY BLADDER BY INORGANIC MERCURY: TIME DEPENDENCE DUE TO APPARENT INACTIVATION OF $Hg^{++}$.

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The nephrotoxic effects of mercury are well documented, but the cellular mechanisms that underlie impairment of renal function are poorly understood. The winter flounder urinary bladder actively secretes K and provides an important model for the study of this aspect of kidney function. Active K secretion by the urinary bladder of the flounder can be measured as a short circuit current ($I_k$) in vitro (Bull. M.B.I.D.L. 20:89-92, 1980). A previous study employed reversible blockers (barium and lidocaine) to induce fluctuations or "noise" in $I_k$ and provided direct evidence for the presence of K channels in the apical membranes of the epithelial cells (Bull. M.D.I.B.L. 25:1-3, 1985). These K channels presumably mediate K exit from the epithelial cells into the lumenal bath. Chang, Betz and Dawson (Bull. M.D.I.B.L. 25:44-45, 1985) presented preliminary data suggesting that inorganic mercury was a potent, reversible blocker of apical K channels in the flounder urinary bladder. The object of the present study was to characterize the block of $I_k$ by $HgCl_2$. We found that the addition of $HgCl_2$ (1 μM.) to the mucosal bathing solution of a tissue mounted in a conventional Ussing chamber produced a transient inhibition of $I_k$ (3-5 min.), but this decline was followed by a complete recovery (15-30 min.)[1]. We tested the hypothesis that the transient block of $I_k$ by $HgCl_2$ was due to inactivation of $Hg^{++}$, possibly by binding or complexation. We found that the partial replacement or constant perfusion of the $HgCl_2$-containing mucosal bathing solution eliminated the recovery of $I_k$, supporting the notion that the $Hg^{++}$ was somehow inactivated in the chamber.

Urinary bladders were removed from flounder and mounted as flat sheets in plexiglass chambers as previously described (Bull. M.D.I.B.L. 19:46-49, 1979). Conventional Ussing chambers (A=1.25 $cm^2$) were used in the first series of experiments and chambers modified for continuous perfusion (A=1.25 $cm^2$) were used thereafter. Tissues were bathed on both sides with identical solutions containing (in mM.) Na:147.5, Cl:147.5, K:2.5, Ca:1.5, Mg:1.0, glucose:5.0 and HEPES:15 (pH=7.5). The serosal solutions also contained verapamil (10 μM.) to inhibit spontaneous contraction of smooth muscle. Mucosal and serosal solutions were stirred with air (Ussing chambers) or by constant fluid flow (perfusion chambers). The short circuit current ($I_k$) was measured as previously described (Bull. M.D.I.B.L. 20:89-92, 1980).

Figure 1 shows the results of a representative experiment in which the effect of $Hg^{++}$ on $I_k$ was compared to that of $Ba^{++}$, a reversible blocker of K channels (Bull. M.D.I.B.L. 25:1-3, 1985). The experiment was conducted using conventional Ussing chambers and two tissues were obtained from a single urinary bladder. The addition of $BaCl_2$ (2 mM) to the mucosal bath of one tissue (solid line) rapidly reduced $I_k$ to near zero followed by a small recovery, in accord with previous reports (Bull. M.D.I.B.L. 20:89-92, 1980). In other tissues, raising the mucosal

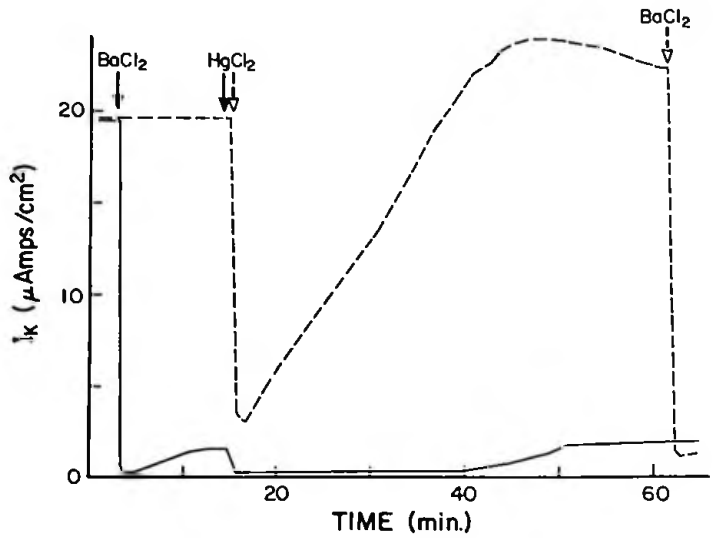


figure 1.

barium concentration to 4 mM. reduced $I_k$ to zero (not shown). The addition of $HgCl_2$ (1μM.) to the mucosal bath of the second tissue (dashed line) rapidly reduced $I_k$ but this was followed by complete recovery over the ensuing 25 minutes. The recovered short circuit current was abolished by mucosal barium confirming that it was due to active K secretion. In the presence of mucosal barium (solid line) the only effect of $HgCl_2$ was to abolish the small residual $I_k$ although this effect also waned after about 30 minutes.

Results such as these suggested to us that inorganic mercury was a potent blocker of apical K channels but that the active form of $Hg^{++}$ may be rapidly inactivated or bound, thus relieving the block of $I_k$. In order to test this hypothesis, we modified the experimental paradigm in two ways to ensure that the mucosal $Hg^{++}$ concentration would remain constant. First we employed a 5 ml. for 5 ml. substitution paradigm wherein one-half the volume of the mucosal bath (5 ml.) was periodically replaced by a fresh solution containing the same concentration of $HgCl_2$. Second, we used chambers that were modified for constant perfusion of the tissue with solutions containing known concentrations of $HgCl_2$. Figure 2 shows the results from two representative experiments. Two tissues were obtained from the urinary bladders of different flounder. One tissue (dashed line) was mounted in a conventional Ussing chamber while another (solid line) was mounted in the chamber modified for the constant perfusion. The y-axis of figure 2 was normalized to allow a better comparison between the two tissues. Maximum values (100%) of $I_k$ for tissues represented by the dashed line and the solid line were 12.8 and 14.4 $\mu A/cm^2$. respectively. At the time indicated, $HgCl_2$ (1 μM.) was added to the mucosal solutions of both tissues. The "♦" indicates times at which 5 ml. of the mucosal bathing solution were removed from the Ussing chamber and replaced with 5 ml. of an identical solution containing 1 μM. $HgCl_2$. Note that either serial replacements or constant prefusion eliminated the recovery of $I_k$. This result supports the hypothesis that $Hg^{++}$ was somehow inactivated. In the perfusion chamber, the block of $I_k$ by $Hg^{++}$ was completely

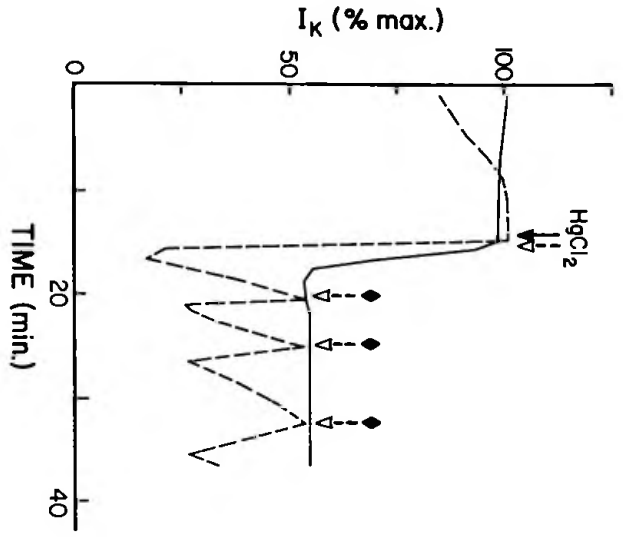


figure 2.

reversed simply by perfusing the mucosal side with a solution that contained no $HgCl_2$ (data not shown) suggesting that the block of $I_k$ by inorganic mercury did not involve a covalent bond.

Figure 3 shows a log dose-response of $I_k$ to mucosal $HgCl_2$ obtained from a single tissue continuously perfused on the mucosal side with increasing concentrations of $HgCl_2$. Percent inhibition was calculated from the steady-state values of $I_k$ 5-10 min. after changing solutions. Table 1 compares the maximum percent inhibition of $I_k$ produced transiently by 1μM. $HgCl_2$ in conventional Ussing chambers to the steady state inhibition produced by the same concentration of $HgCl_2$ in perfusion chambers. In the perfusion chambers 1 μM. $HgCl_2$ reduced $I_k$ by 55%. In Ussing chambers, the same average percent inhibition was obtained (56%) but with a much greater degree of variability. Note that

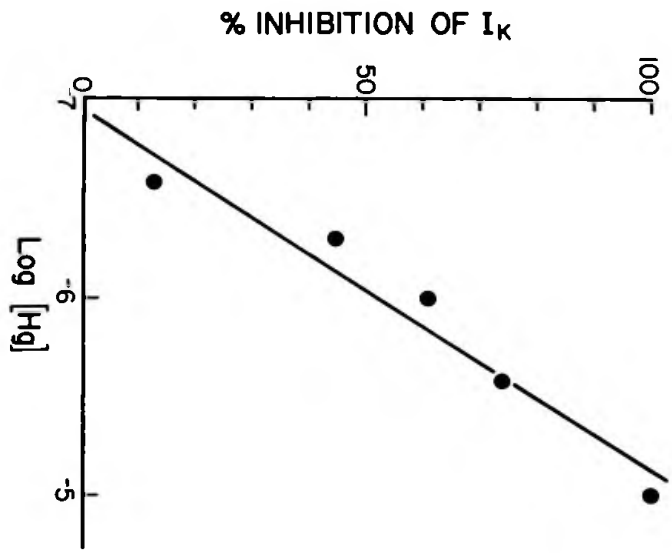

Table 1. Transient Versus Steady State Inhibition of $I_k$ by 1 μM. $Hg^{++}$

| | % Inhibition of $I_k$ | S.E. | n |
|---|---|---|---|
| Perfusion chambers | 55% | ±10.4% | 3 |
| Ussing chambers | 56% | ±34.8% | 12 |

the results summarized in table 1 are in close agreement with that shown in figure 3. These data suggest that $Hg^{++}$ is a very potent blocker of $I_k$ with an effective concentration for 50% block ($EC_{50}$) of less than 1 μM. Additional experiments (not shown) indicated that at higher concentrations (5-10 μM.), $Hg^{++}$ appeared to cause some relatively nonspecific increases in apical membrane ion permeability, a result in accord with the well-known permeabilizing effects of mercurials (Ann. Rev. Biochem., 41:91, 1972, Pharm. Rev., 13:185,1961, Toxicol. Appl. Pharmacol., 37:339-348, 1976).

These data provide additional support for the notion that $Hg^{++}$ is a potent, reversible blocker of apical K channels in the flounder urinary bladder. The $EC_{50}$ was less than 1 μM. or 200 parts per billion (ppb.) and partial blockade could be produced with as little as 0.2 μM. (40 ppb.). This is to be compared to barium which exhibits an $EC_{50}$ of about 50 μM. (unpublished results). The $Hg^{++}$ block was readily reversed by washing suggesting that the action of this ion may be similar to that of barium ie: the ion may enter the channel and occlude the pore (Vergara, C. and R. Latorre. J. Gen. Physiol. 82:543-568, 1983).

These experiments also suggest that $Hg^{++}$ can be rapidly inactivated, possibly by complexation, thus relieving the block of $I_K$. Wilbrandt described a time dependence for the action of inorganic mercury on glycerol uptake in red blood cells (Pflugers Arch. 244:637-43, 1941). $HgCl_2$ inhibited glycerol uptake temporarily and after a lag period, the cell membrane regained its original permeability. Mercury forms complexes with many organic molecules including; cysteine, histidine, oxaloacetate, purine bases, pyrimidine bases, phosphates and orthophosphates (for a review see Trace Metals Exposure and Health Effects, E.D. Ferrante ed., pp.73-98, Pergamon Press, Oxford, 1979). Because of the high affinity of SH groups and nucleotide bases for $Hg^{++}$ (pK = 20-23, ibid.), it is likely that the mercury was inactivated by complexation. These results indicate that caution must be exercised in designing experiments to test the effects of $Hg^{++}$ in vitro.

Finally, the high affinity of $Hg^{++}$ for organic groups common to biological tissues makes it all the more remarkable that the ion is a reversible blocker of a channel protein. The reversibility of the $Hg^{++}$ block may argue against the presence of certain functional groups (eg: sulfhydryls) in the conducting region of the channel. (Supported by grants from NIADDK and NIEHS).

[1]In a previous study of inhibition of $I_k$ by $Hg^{++}$ the recovery did not come to our attention because exposures were limited to short times. Unfortunately, this was not clear in our previous report (Bull. M.D.I.B.L. 25:44-45, 1985) because the abscissa of figure 1 was incorrectly labeled as 180 minutes full scale rather than 30 minutes.