INHIBITION OF HUMAN CFTR BY Hg: ANION DEPENDENCE

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The propensity of metals, like mercury (Hg), to form polyatomic, anionic complexes in aqueous solution is well known (Dawson, D.C. and Ballatori, N., *Handbook of Experimental Pharmacology*, Vol. 115, pp. 53-72, 1995). In previous experiments (Smith, S.S., et al., *Ped. Pulmonol. Supp.* 14, p. 220, 1997) we showed that members of a family of polyatomic anions, the pseudohalides, are potent blockers of the cystic fibrosis transmembrane conductance regulator (CFTR). The goal of the experiments described here was to determine if a polyatomic complex involving Hg would also block CFTR.

All of the experiments were carried out using human CFTR expressed in *Xenopus* oocytes as previously described (Wilkinson, D.W., et al., *J Gen Physiol..*, 107: 103-119, 1996). The properties of CFTR chloride channels were assayed by means of a two-electrode voltage clamp. Current-voltage relations were obtained periodically using a command potential that was ramped at a constant rate of 100 mV/sec from −120 to +60 mV. Oocytes were initially perfused with an amphibian Ringer's solution containing in mM, Na: 100.5, K: 1.8, Ca: 1.8, Mg: 1.0, Cl: 105.6, HEPES: 5 at pH 7.5. After the membrane conductance had stabilized, the perfusion solution was supplemented with forskolin (10 μm) and isobutylmethylxanthine (IBMX, 1 mM) in order to activate CFTR chloride channels (Wilkinson, D.W., et al., *J. Gen Physiol*). After the activated CFTR conductance had stabilized, the oocyte, in the presence of the activating solution, was exposed to $HgCl_2$ or NaSCN in various combinations. All records were corrected for the background conductance of the oocyte determined prior to the activation of CFTR.

Figures 1 and 2 contain representative I-V plots demonstrating that the simultaneous presence of Hg and SCN produces a profound inhibition of CFTR conductance that is not seen with either ion alone. In Figure 1, the solid line represents the I-V plot obtained after activation of CFTR in amphibian Ringer's. The dotted line is the plot obtained after a 5-minute exposure to amphibian Ringers plus 1 mM NaSCN. The polyatomic anion produced, at this concentration, a modest but readily discernible inhibition of CFTR conductance. Subsequent exposure of the oocyte to 10 μM $HgCl_2$ in the presence of SCN, however, inhibited the CFTR conductance by about 40%. Figure 2 illustrates the results of a similar experiment in which the order of addition of SCN and Hg was reversed. It can be seen that HgCl alone produced only a modest inhibition of CFTR conductance whereas the simultaneous presence of Hg and SCN markedly reduced CFTR conductance, in this case by about 50%. The effect of the Hg was not readily reversed by washing with amphibian Ringer's.

The results are consistent with the hypothesis that Hg and SCN can combine in solution form a polyatomic complex that is a potent inhibitor of CFTR. Polyatomic anions are predicted to enter the CFTR pore readily because they are less tightly held by their waters of hydration than is chloride. In addition, such anions have been demonstrated to bind tightly in the CFTR pore so that anionic throughput is blocked. Hg is predicted to form complexes with SCN in solution (Cotton, F.A., and Wilkinson, G., *Advanced Inorganic Chemistry*, 8th ed., p 612, John Wiley and Sons, 1988) and these may enter the pore and bind as do other polyatomic species such as $Au(CN)_2$ and $C(CN)_3$ (2). It is important to note that although the concentration of $HgCl_2$ producing approximately half-maximal inhibition was 10 μm, the concentration of the, as yet unidentified, active species is expected to be considerably less due to the competition for the formation of polyatomic, Hg-containing species from SCN, Cl, OH and HCO_3 anions. The results suggest that the

toxic effects of exposure to Hg could be exacerbated in the presence of anions like SCN which are abundant in the blood of smokers (Goodman & Gilman, *The Pharmacological Basic of Therapeutics*, 9th ed., p. 1401, McGraw-KHill, 1996). Supported by NIEHS (P30 ES03828), NIH (DK45880,DCD), and NSF (DBI9531348).

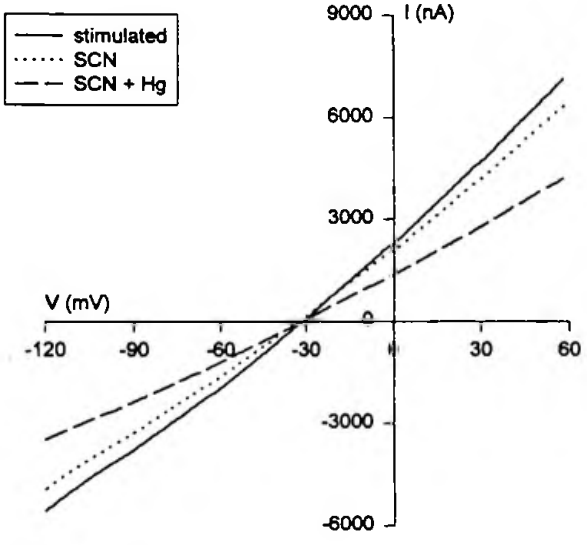

FIGURE 1
I-V relation for CFTR under control conditions, after exposure to 1 mM SCN and after exposure to 1 mM SCN + 10 μM $HgCl_2$

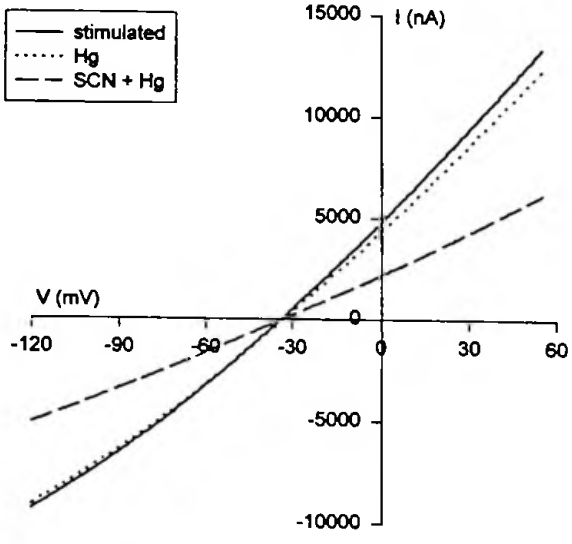

FIGURE 2
I-V relation for CFTR under control conditions, after exposure to 10 mM $HgCl_2$ and after exposure to 10 μM $HgCl_2$ + 1 mM SCN