SHARK AND HUMAN CFTR EXPRESSED IN *XENOPUS* OOCYTES HAVE DIFFERENT SENSITIVITES TO INHIBITION BY THE THIOL-REACTIVE METALS MERCURY AND ZINC

Jeffrey C. Sirota[1,2], Gerhard J. Weber[1,3], Stephen G. Aller[1], David C. Dawson[4], and John N. Forrest, Jr.[1]
[1]Dept. of Medicine, Yale University School of Medicine, New Haven, CT 06510
[2]Yale College, New Haven, CT 06510
[3]Experimentelle Nephrologie, Domaglzstr 3a, Münster, Germany 48149
[4]Dept. of Physiology, University of Michigan Medical School, Ann Arbor, MI 48109

Recent perfusion and short-circuit current studies have indicated that the apical membrane is an important site of mercury toxicity in shark rectal gland cells and that shark CFTR may be a target for Hg (Ratner, M. et al., *Bull. MDIBL* 37:20-21, 1998). In further studies, we have now compared the inhibitory effects of mercury on shark and human CFTR in the *Xenopus* oocyte heterologous expression system. Additionally, we have explored differences in the sensitivity of these CFTR isoforms to the heavy metal zinc.

The effects of mercury and zinc were studied using two-electrode voltage clamping of microinjected *Xenopus laevis* oocytes. Mature oocytes were isolated, manually defolliculated, and injected with human CFTR (hCFTR) or shark CFTR (sCFTR) cRNA. Oocytes were incubated at 18 °C for thirty-six hours before experiments. After the oocyte reached a stable baseline conductance in Frog Ringer's solution (98 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 2.5 mM HEPES Na salt, 2.5 mM HEPES acid, pH 7.4), chloride conductance was stimulated by perifusing with 10 μM forskolin and 1 mM isobutylmethylxanthine (IBMX). Oocytes were stimulated for 50 min to achieve a steady-state conductance (80 – 140 μS). Potential metal inhibitors were then added, and the percent inhibition was determined 30 min later.

As shown in figure 1 (Panels A and C), shark CFTR is markedly sensitive to inhibition by mercuric chloride. In oocytes expressing shark CFTR, chloride conductance was inhibited ~ 70% by 1 μM mercuric chloride, with an IC_{50} of ~ 0.8 μM (Panel C). In contrast, despite comparable stimulation of conductance by forskolin and IBMX (Panel B), human CFTR is insensitive to 1 μM mercuric chloride. Maximum inhibition of human CFTR by mercuric chloride was ~15% at the highest concentrations used (Panel C). Dithiothreitol (DTT, 100 μM) added simultaneously with $HgCl_2$ entirely prevented the inhibitory response, and DTT added after mercurial inhibition markedly reversed the $HgCl_2$ effect (data not shown).

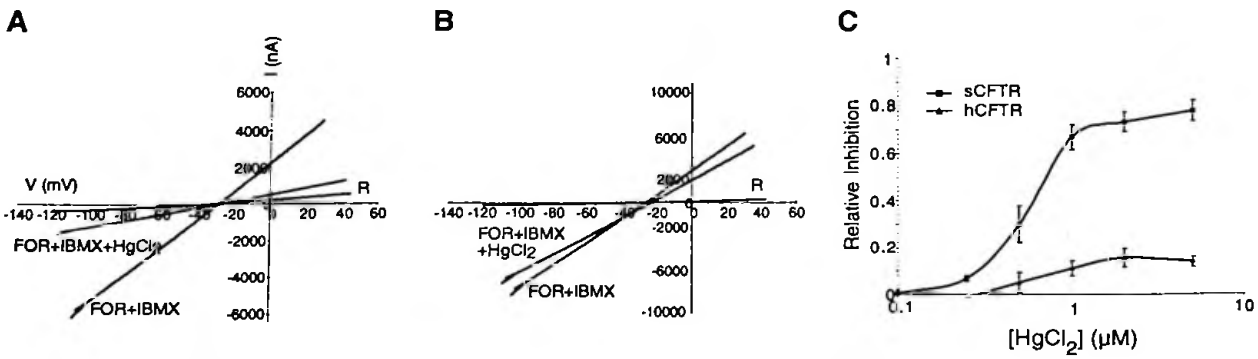

Figure 1. Inhibition of shark CFTR and human CFTR by $HgCl_2$. Panel A: I-V plot from an oocyte expressing shark CFTR. Panel B: I-V plot from an oocyte expressing human CFTR. For panels A and B, I-V plots are given for Ringer's only (R), FOR+IBMX, and FOR+IBMX+$HgCl_2$ (1μM). Panel C: Dose response to mercury inhibition of shark vs. human CFTR. (n = 3 to 9 oocytes per concentration).

We also examined the effects of a second thiol-reactive metal, zinc. Using the same protocol, we compared the effects of zinc acetate on FOR+IBMX stimulation of shark vs. human CFTR. In concentrations from 50 – 200 μM, zinc acetate markedly inhibited shark CFTR by 40-75% (Figure 2, Panels a and C). These concentrations of zinc were essentially without effect (< 5% inhibition) on human CFTR (Figure 2, panels B and C). The effect of zinc was promptly reversed by washout of the metal with frog Ringer's containing FOR+IBMX.

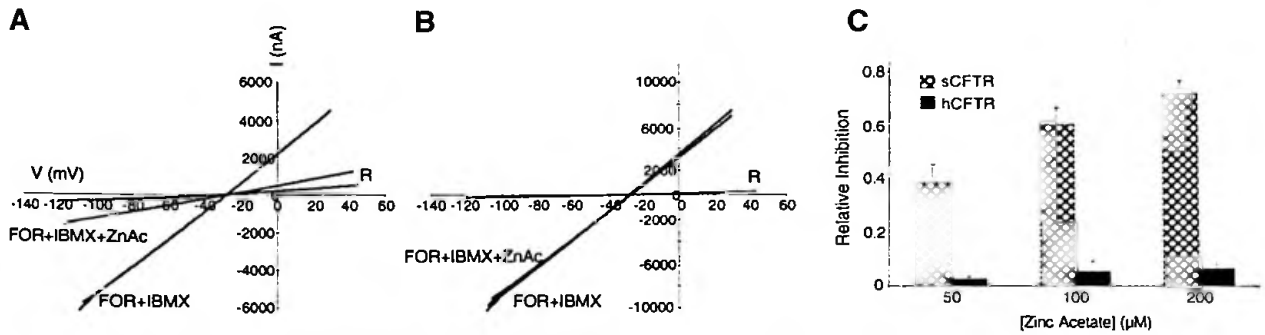

Figure 2. Inhibition of shark CFTR and human CFTR by zinc acetate. Panel A: I-V plot from an oocyte expressing shark CFTR. Panel B: I-V plot from an oocyte expressing human CFTR. For panels A and B, I-V plots are given for Ringer's only (R), FOR+IBMX, and FOR+IBMX+ZnAc. Panel C: Dose response to zinc inhibition of shark vs. human CFTR. (n = 3 to 4 oocytes per concentration).

These experiments demonstrate a profound difference in the sensitivity of shark vs. human CFTR to inhibition by the thiol-reactive metals, mercury and zinc. At comparable conductances activated by forskolin and IBMX, shark CFTR is inhibited ~ 70% in the presence of mercury and zinc, whereas these metals have minimal inhibitory effects on the human CFTR homolog. The exquisite sensitivity of shark CFTR to mercury (IC_{50} of ~ 0.8 μM) suggests that this protein is a major site for the toxic effects of mercury in this species. An analysis of cysteine (Cys) residues in shark and human CFTR indicate that the proteins share 12 cysteine residues, including 3 in transmembrane domains. The shark homolog has 6 unique Cys not present in human, including 2 in transmembrane regions and 2 in the R-domain. We consider these Cys residues to be potential sites for inhibition by thiol-reactive metals (mercury and zinc) and are currently conducting site-specific mutagenesis of these residues to examine this hypothesis.

This work was supported by NIH grants DK 34208 and NIEHS P30-ES 3828 (Center for Membrane Toxicology Studies), a Grant in Aid from the American Heart Association, Maine Affiliate, and NSF Dorm grant DBI-9714056.