EFFECT OF MERCURY ON Ca^{2+}-ACTIVATED CHLORIDE CURRENTS

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Pollution of our seas and waterways with mercury is a serious environmental problem, but the molecular mechanisms of mercury toxicity remain poorly understood. Because ion channels are trans-membrane proteins having extra-cellular thiol groups that are accessible to environmental mercury, ion channels are a prime target for mercury toxicity. Previous investigators at MDIBL have demonstrated that CFTR, a Cl channel responsible for salt balance in sharks, is exquisitely sensitive to mercury compared to mammalian CFTR (Sirota et al., *MDIBL Bulletin* 38:105-106, 1999). In this investigation, we examined the sensitivity of Ca^{2+}-activated Cl^- channels to mercury. Ca^{2+}-activated Cl^- channels play important roles in epithelial salt and water transport in many cell types (Fuller (ed), *Ca^{2+}-activated Cl^- Channels,* Academic Press, 2001). We have proposed that Ca^{2+}-activated Cl^- channels may be encoded by the bestrophin family of proteins (Hartzell et al, *submitted*). This family has 2 highly conserved cysteine residues that face the extra-cellular space that may be susceptible to thiol-reactive reagents such as mercury. In these experiments, we examined the effects of Hg on Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes. We found that, in contrast to CFTR, Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes are relatively insensitive to Hg. *Xenopus* oocytes were voltage-clamped with two micro-electrodes and Ca^{2+}-activated Cl^- currents were elicited by microinjection of 10 nl of 10 mM inositol 1,4,5-trisphosphate (IP_3) into the oocyte as we have previously described (Hartzell, *J. Gen. Physiol.* 108:1-19, 1996). On average, IP_3 injection elicited Ca^{2+}-activated Cl^- currents ~10 μA in amplitude at +80 mV. Figure 1 shows that there was no effect of Hg on the Ca^{2+}-activated Cl^- currents at concentrations between 10 μM (left panel) and 100 μM (right panel). Hg had no significant effect on the background Ca-insensitive Cl currents before IP_3 injection.

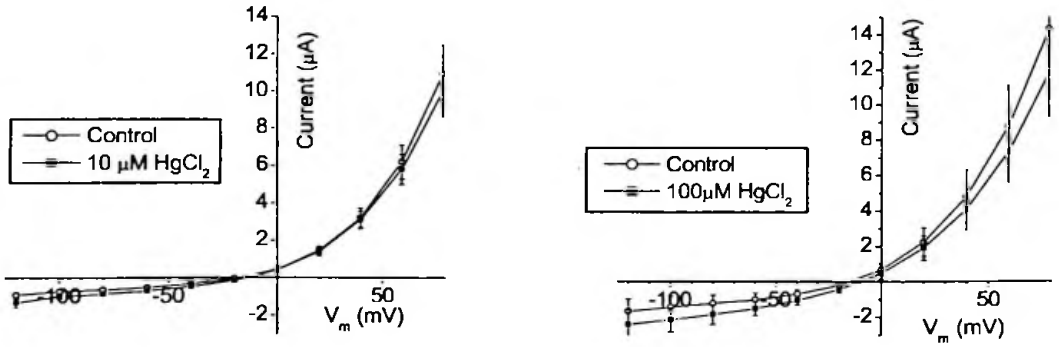

Figure 1. Absence of effect of $HgCl_2$ on Ca-activated Cl currents in *Xenopus* oocytes. Xenopus oocytes were voltage-clamped with two microelectrodes using an Axon Instruments Gene Clamp 500B amplifier. The bath was grounded via a Ag-AgCl electrode

attached to a virtual ground bath clamp (Axon Instruments). The cells were held at a membrane potential of -40 mV and stepped to various potentials between -120 mV and +80 mV for 1-sec. Ca-activated Cl currents were elicited by injection of 10 nl of 10 mM IP_3. After 10 min, when the Ca-activated Cl currents had reached a maximum amplitude, the peak current at each voltage was plotted. The left panel shows the average current-voltage relationships of 10 oocytes before (open symbols) and during (solid symbols) exposure to 10 μM $HgCl_2$. The right panel shows the average current-voltage relationships of 8 oocytes before (open symbols) and during (solid symbols) exposure to 10 μM $HgCl_2$.

Supported by the Center for Membrane Toxicity Studies and the Hancock County Scholars Program.