MICROMOLAR CONCENTRATIONS OF INORGANIC MERCURY ALTER MEMBRANE CONDUCTANCE OF XENOPUS OOCYTES

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In the course of experiments in which Xenopus oocytes were employed as an expression system for the study of the effects of inorganic mercury on cloned transporters, we investigated the effects of mercury on the background ionic permeability of the oocyte plasma membrane. Oocytes were removed from frogs and defolliculated as previously described (L.S. Smit, D.J. Wilkinson, M.K. Mansoura, F.S. Collins, and D.C. Dawson, Proc. Nat. Acad. Sci. 90:9963-9967, 1993). During experiments oocytes were perfused with a modified Barth's solution that contained (in mM): 98 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$ and 5 Hepes (pH 7.4, 220 mOsm). A two electrode voltage clamp was used to monitor membrane conductance. Current-voltage relations for the oocyte membrane were obtained by means of a ramp command that varied the clamping potential from -120 mV to +60 mV over a two second interval. The holding potential was generally -60 mV. Currents and voltages were acquired by means of an IBM compatible computer using "pCLAMP" software (Axon Inst, Foster City, CA).

Figure 1 shows a representative I-V plot for an oocyte under control conditions and after exposure to 1 μM $HgCl_2$ for 9 min. Under control conditions the I-V plot for the oocyte exhibited a characteristic appearance. At negative membrane potentials the plot was linear with a reversal potential of about -30 mV. At positive potentials the curve was S-shaped due to the activation of an endogenous, calcium-activated, chloride-selective conductance (D.J. Wilkinson, M.K. Mansoura, P.Y. Watson, L.S. Smit, F.S. Collins, and D.C. Dawson, J. Gen. Physiol. 107:103-119, 1996). Exposure of the cell to 1 μM $HgCl_2$ in frog Ringer's, shifted the reversal potential toward more negative values and increased the slope conductance at the reversal potential. The effect of $HgCl_2$ was not reversed by washing the oocyte with $HgCl_2$-free frog Ringer's and was only partially reversed by the application of 100 μM dithiothreitol.

The Effect of 1μM $HgCl_2$ on Whole Cell Conductance

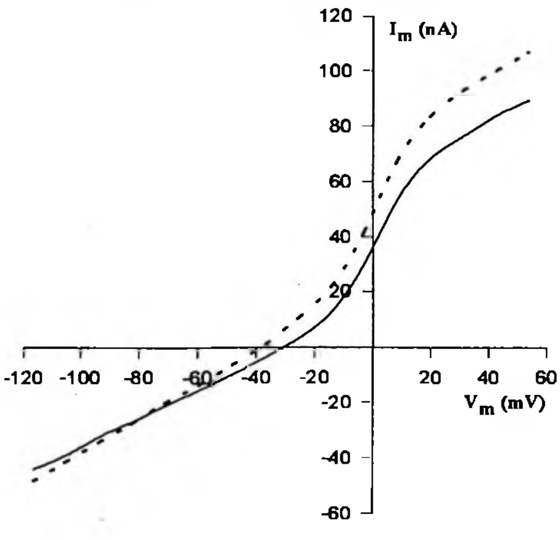

FIGURE 1. Current-voltage plots for Xenopus oocyte in the presence of frog Ringer's (solid line) and after exposure to 1μM $HgCl_2$ in frog Ringer's (dashed line). In the absence of $HgCl_2$, the membrane current reversed at -33.2mV and the slope conductance at the reversal potential was 0.591μS. Exposure to $HgCl_2$ shifted the reversal potential to -39.1mV and increased the slope conductance to 0.712μS.

These results indicate that micromolar quantities of $HgCl_2$ can significantly alter the ionic conductance of the oocyte plasma membrane. The increase in slope conductance and leftward shift in the reversal potential are compatible with the hypothesis that the target for $HgCl_2$ was a population of potassium-selective channels. The observed response could be accounted for by increases in either the single channel conductance or the open probability of such channels, or by some combination of these effects. These findings suggest that micromolar concentrations of $HgCl_2$ might compromise cellular processes like volume regulation by activating potassium channels. In addition, the effect of Hg^{2+} on endogenous oocyte potassium channels could complicate any evaluation of the metal's action on cloned channels expressed in oocytes (Supported by NIEHS E503829).