EFFECT OF Hg^{2+} ON THE TRANSMEMBRANE POTENTIAL OF RED BLOOD CELLS FROM GLYCERA DIBRANCHIATA

Robert Wondergem[1] and Chad G. Sisson[2]
[1]Department of Physiology, James H. Quillen College of Medicine
East Tennessee State University, Johnson City, Tennessee 37614
[2]The Mount Desert Island Biological Laboratory, Salsbury Cove, Maine 04672

Our interest has been to assay rapid effects of mercuric ion on the transmembrane potential (V_m) of either cultured epithelial cells or red blood cells in vitro. We showed previously that Hg^{2+} at high concentrations (10^{-4} & 10^{-5} M) rapidly depolarized V_m of cultured cells obtained from shark rectal gland; whereas, at lower concentration (10^{-6} M) Hg^{2+}-induced depolarization of V_m was delayed by an average of 52 min (Wondergem, R. and K.S. Davis, Bull. Mt. Desert Isl. Biol. Lab. 28: 90-91, 1989.). Preston and Chen recently demonstrated that Hg^{2+} (20 μM) rapidly (< 1 min) decreases Na-dependent taurine transport into coelomocytes (red blood cells, RBC's) of the marine polychaete bloodworm, Glycera dibranchiata (Preston, R. and C. Chen, Bull. Environ. Contam. Toxicol. 42: 620-627, 1989). Our objective was to measure effects of Hg^{2+} on V_m of Glycera RBC's and, thereby, to discern whether the inhibitory effect of Hg^{2+} on taurine transport into RBC's was a direct effect on the membrane carrier or an indirect effect caused by depolarization of V_m and decrease in the transmembrane electrochemical Na^+ gradient. The latter provides electrochemical force for secondary active transport of taurine into Glycera coelomocytes.

Glycera red blood cells in artificial sea water (ASW) were allowed to settle onto plastic coverslips for 30 min. These were transferred to a perfusion chamber on the stage of an inverted microscope. ASW comprised (in mM): 440 NaCl, 9 KCl, 9.3 $CaCl_2$, 23 $MgCl_2$, 26 $MgSO_4$, 2.2 $NaHCO_3$, and pH was 7.8. Microelectrodes were pulled from fiber-filled, borosilicate glass capillaries, and they had tip-resistances of approximately 100 MΩ when filled with 0.5 M KCl. Microelectrode input resistance was measured by passing intermittent current pulses (0.1 nA, 300 ms duration) through the microelectrode after compensating resistance of the latter. A micromanipulator, which was attached to the microscope stage and controlled by a remote joystick, positioned microelectrodes immediately above the RBC's. They were advanced until they touched the cells as discerned by cell movement, by slight shifts in offset voltage, and by increases in microelectrode input resistance. Cell impalement was achieved by lightly tapping the table supporting the microscope.

V_m of bloodworm RBC's was -93 ± 1.8 mV (SE; n = 31), and this was in good agreement with previous findings (Wondergem, R., and R. Preston, Bull. Mt. Desert Isl. Biol. Lab. 26: 103-104, 1986). Complete substitution of extracellular Cl^- with gluconate had no effect on V_m. However, increasing external $[K^+]$ by substituting NaCl with K gluconate resulted in rapid, reversible depolarization of V_m. Steady-state V_m was plotted vs $\log_{10} [K^+]_o$ ranging from 4.5 to 440 mM, and the slope was -53 mV per tenfold change in $[K^+]_o$. This was in good agreement with the Nernstian relationship expected for a K^+-selective electrode, and this showed that membrane K^+ conductance in Glycera RBC's is the primary determinant of V_m. These measurements also showed that Glycera RBC V_m can be

measured readily with glass microelectrodes in spite of their small size and absence of attachment to a substratum. Success required very fine-tipped microelectrodes and dense settling of cells onto a coverslip, which minimized cell movement on impalement with the micropipette.

In three separate cells, 20 μM Hg^{2+} had no effect on RBC V_m over period of exposure to the heavy metal ranging from 8 to 33 min. In one cell, Hg^{2+} at this concentration depolarized V_m from -96 to -68 mV, but this decrease in V_m began only after a 6 min exposure to Hg^{2+}. In three separate cells, 200 μM Hg^{2+} depolarized RBC V_m from -79 ± 4 mV to -19 ± 0.3 mV (n = 3), and the onset of these decreases in V_m ranged from 6 to 23 min after exposure to Hg^{2+}. None of these effects of Hg^{2+} on V_m were reversible. These findings show that Hg^{2+} inhibits membrane function in Glycera RBC's. Nevertheless, these inhibitory effects of Hg^{2+} have a prolonged time course of onset compared with that for inhibition of taurine transport at similar concentrations (Preston, R. and C. Chen, Bull. Environ. Contam. Toxicol, 42: 620-627, 1989). Our findings rule out membrane depolarization and dissipation of the transmembrane electrochemical Na^+ gradient as the explanation of inhibition of taurine transport by Hg^{2+}.

Heavy metal intoxication is suspected in the etiology of a number of human diseases and disorders, ranging from neurological deficits to nephrosis. For example, mercurial diuretics are contraindicated in presence of renal disease and are seldom used. Nevertheless, mechanisms by which these agents effect cell damage, particularly at the level of membrane function, remain unknown. Current studies present simple and convenient means of assessing effects of heavy metals on membrane function, and thus they serve a useful purpose in assessing the mechanism of toxic effects of heavy metals on cell function. Future studies will utilize patch-clamp techniques to discern whether depolarizing effects of Hg^{2+} on Glycera V_m result from non-specific increases in membrane ion permeability or whether these effects result from specific inhibition by Hg^{2+} of membrane K^+ channels. The near Nernstian response of changes in V_m with changes in external $[K^+]$ suggests that membranes of Glycera RBC's contain primarily K^+ channels and therefore may be well suited to studying effects of heavy metals on membrane channel function.

The authors thank R. Preston for providing Glycera dibranchiata for this study. This work was supported by NIEHS-ESO3828-05 to the Center for Membrane Toxicity Studies and the Hearst Foundation Summer Scholarship Program.