MERCURY INHIBITION OF Na/K ATPase IN XENOPUS LAEVIS OOCYTES

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Na/K ATPase is a target for mercurials and other sulfhydryl reactive compounds (A. Schwartz, G.E. Lindenmayer, and J.C. Allen, Pharm. Rev. 27(1):3-134, 1975). Recent studies suggest that the action of mercury on Na/K ATPase may account for a significant portion of the toxicity of this compound (N. Ballatori, C. Shi, and J.L. Boyer, Tox. and App. Pharm. 95:279-291, 1988; E. Imesch, M. Moosmayer, and B.M. Anner, Am. J. Physiol. 262: F830-F836; F837-F842, 1992). Most studies of mercurial interaction with Na/K ATPase have been conducted using isolated enzyme (G.R. Henderson, W.H. Huang, and A. Askari, Biochem. Pharm. 28:429-433, 1979), or enzyme reconstituted in liposomes (B.M. Anner and M. Moosmayer, Am. J. Physiol. 262:F843-F848, 1992). We sought to evaluate the possible effect of mercury on Na/K ATPase under physiological conditions. We used Xenopus oocytes to study the effect of mercury on the 'pump current' generated by electrogenic Na/K exchange.

Oocytes were removed from mature frogs and manually defolliculated as previously described (L.S. Smit et al., Proc. Nat. Acad. Sci. 90:9963-9967, 1993). Oocytes were maintained in modified Barth's Ringer which contained (in mM): 98 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$ and 5 HEPES (pH 7.4; 220 mOsm). During an experiment the oocyte was continuously perfused. A two electrode voltage clamp (Dagan TEV-200) was used to maintain the holding potential at -60mV, and current-voltage relationships were generated by applying a voltage ramp from -120mV to +60mV over a two second interval. The holding current was continuously monitored on a strip chart recorder and voltage ramps were generated and the resulting currents acquired using an IBM compatible computer running pClamp software (Axon Instruments, Foster City, CA). We used the technique of A. Sagar and R.F. Rakowski (J. Gen. Physiol. 103:869-894, 1994) to measure the current due to electrogenic Na/K exchange. To stimulate pump turnover oocytes were Na loaded by incubation in isosmotic Na citrate (54mM) for 30-120 min. The test solutions were designed to minimize non-pump mediated currents and contained (in mM): 90-50 Na-Aspartate, 0-40 K-Aspartate, 20 TEA-Cl, 5 Ba-Acetate, and 5 HEPES (pH 7.4). The pump current was operationally defined as that current which was activated by external K and inhibited by ouabain.

Figure 1

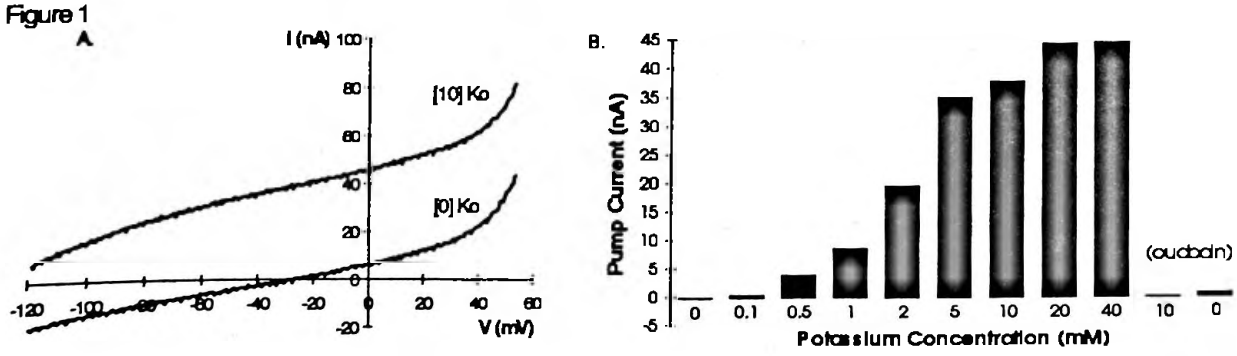

Figure 1A shows I-V plots for a representative, Na-loaded oocyte in the presence and absence of external K. Over this voltage range the Na/K pump current (defined as the difference between the two curves) was fairly

constant, suggesting that over much of the voltage range the pump behaves as a constant current source. Panel B shows the dose response in the same oocyte for activation of pump current by $[K]_o$ at V_m = -40mV. The last two bars in panel B show that the current was abolished by ouabain (1mM). Generally, the non-pump-K-sensitive current was negligibly small (not shown), however there is some variability between oocytes, particularly at positive potentials.

Figure 2

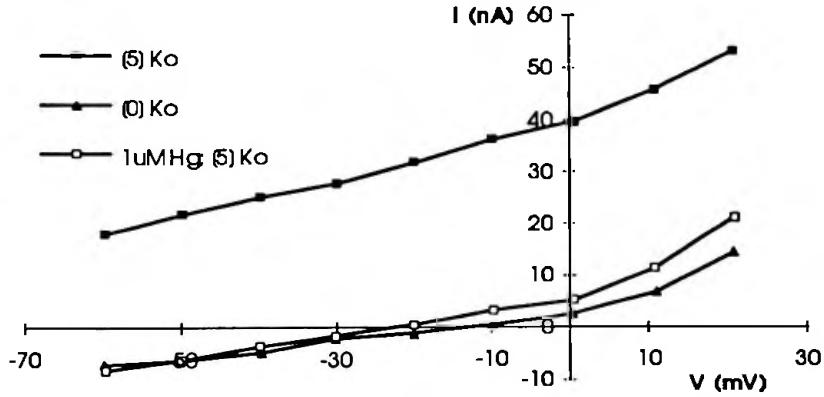

Figure 2 shows that the pump current was inhibited by the addition of 1μM $HgCl_2$. The addition of 1μM $HgCl_2$ in the presence of 5mM K_o resulted in an I-V plot that was superimposable with the 0 K_o curve (not shown is 1mM ouabain in the presence of 5mM K_o, which also superimposes over the 0 K_o curve). The time for complete inhibition varied from one oocyte to another, but was generally complete after 30 minutes. The mercury block was poorly reversed by washing in Hg-free media; the addition of 100 μM dithiothreitol (DTT) to the external perfusate alleviated the block. This observation suggests that the mercury is forming a stable thiol complex with Na/K ATPase, hence the reactive species is probably Hg(II). The dose of Hg which was effective in this setting is an order of magnitude lower than the 'low' dose (10 μM) which was used on isolated or reconstituted enzyme (e.g. B.M. Anner and M. Moosmayer, Am. J. Physiol. 262:F843-F848, 1992). These results suggest that the Xenopus oocyte may be useful model for studying the interaction of inorganic mercury with Na/K ATPase.

This work was supported by the NIEHS center grant #P30-ES03828.