INHIBITION OF CFTR-MEDIATED CHLORIDE CURRENTS IN XENOPUS OOCYTES: COMPARISON OF $HgCl_2$ AND GLIBENCLAMIDE

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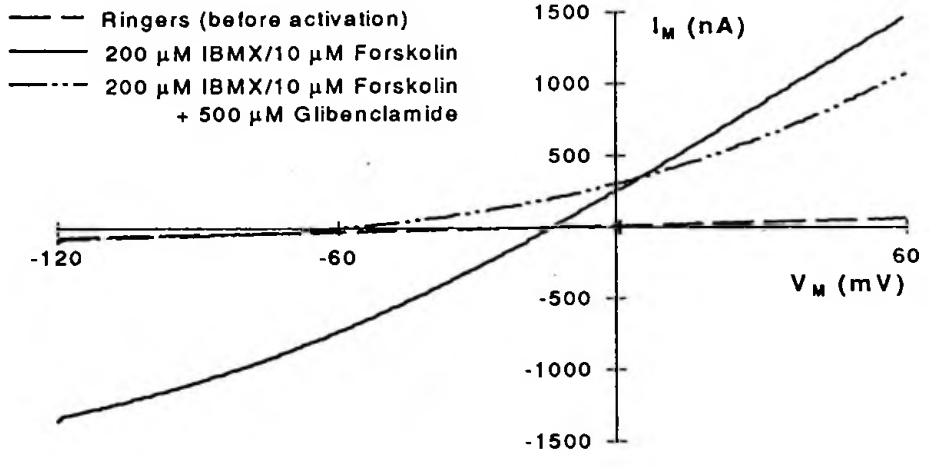
We used Xenopus oocytes expressing the human cystic fibrosis transmembrane conductance regulator (CFTR) to evaluate the possibility that this protein might be a target for inorganic mercury. As a comparison, we used the sulfonylurea compound, glibenclamide, that has been reported (Sheppard, DN and Welsh, MJ, J. Gen. Physiol. 100:573-591, 1992) to inhibit CFTR in NIH 3T3 fibroblasts, HeLa cells, and the mouse mammary cell line, C127; but has not been tested in oocytes expressing CFTR.

Oocytes were removed from Xenopus as previously described (Smit et al., Proc. Natl. Acad. Sci., USA 90:9963-9967, 1993). After removal of the follicular membrane, oocytes were injected with 0.4 ng of mRNA transcribed from a human CFTR cDNA. Cyclic-AMP activated chloride currents mediated by CFTR were assayed by means of a two-electrode voltage clamp. Oocytes were constantly perfused with a standard amphibian Ringers containing, in mM, 98 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES. Mercuric chloride was added to the perfusate from a concentrated aqueous stock solution and glibenclamide was added to the perfusate from a concentrated stock in DMSO.

CFTR chloride currents were activated by perfusing oocytes with a solution containing 10 micromolar forskolin (an activator of adenyl cyclase) and 200 micromolar IBMX (isobutyl methylxanthine, a non-specific phosphodiesterase inhibitor). Current-voltage relations were obtained using a ramp voltage command. Figure 1 contains current-voltage plots for three conditions: a) before activation of chloride current, b) following exposure to forskolin and IBMX, and c) after exposure to 500 micromolar glibenclamide. This concentration of

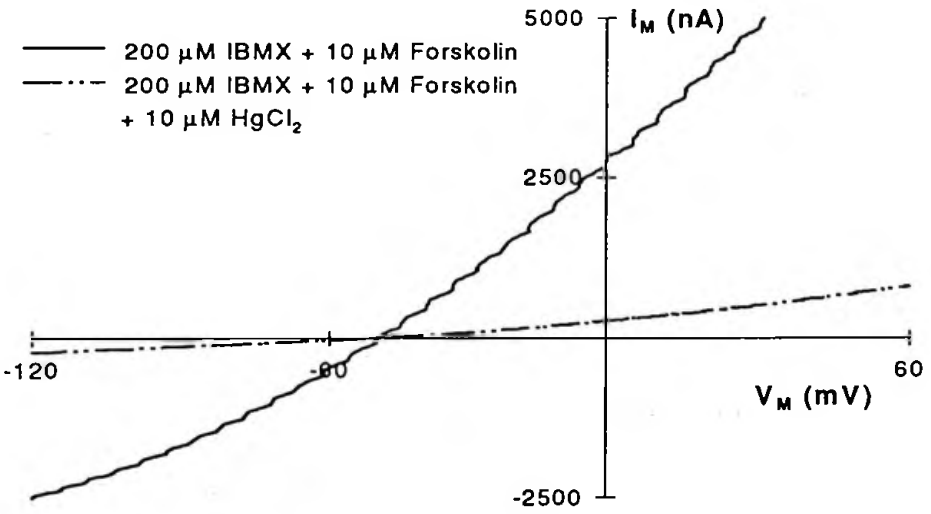
Figure 1 : Glibenclamide Block of CFTR Chloride Current



glibenclamide virtually abolished the inward current, but a substantial outward current remained, suggesting that the action of the compound on the oocyte membrane was complex. The onset of inhibition was relatively slow (3-5 minutes to steady-state) and was reversible, although washout required 15-20 minutes. The apparent $K_{1/2}$ for block by glibenclamide was approximately 150 micromolar.

Figure 2 contains current-voltage plots acquired in the activated state in the absence and in the presence of 10 micromolar $HgCl_2$. This concentration of $HgCl_2$ produced a profound inhibition of the chloride current, but the effect was not reversed by washing with $HgCl_2$-free Ringers. Typically, after prolonged exposure to $HgCl_2$ the oocytes began to show signs of rapid deterioration and the membrane conductance increased markedly.

Figure 2 : Mercury Block of CFTR Chloride Current



These results suggest that exposure to $HgCl_2$ inhibits the cAMP-activated CFTR chloride current, but they shed no light on the mechanism of the effect. Mercury can exist in polyanionic forms in solution and could gain access to the interior of the oocyte via the lipid bilayer or via CFTR itself. The target for mercury could be CFTR itself or some component of the intracellular machinery that functions to activate the channels.

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