EFFECT OF $HgCl_2$ ON ION CURRENTS OF THE ISOLATED HEPATOCYTE OF THE LITTLE SKATE, *RAJA ERINACEA*

G. Robert Jackson, Jr.[1], Horacio F. Cantiello[1], Nazzareno Ballatori[2], James L. Boyer[3]

[1]Renal Unit, Massachusetts General Hospital East, Charlestown, MA 02129, and Department of Medicine, Harvard Medical School, Boston MA 02115
[2]Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, NY 14642
[3]Department of Medicine and Liver Center, Yale University School of Medicine, New Haven, CT 06510

Exposure of cells to a hypoosmotic environment induces volume regulatory mechanisms which move ions across the cell membrane. The hepatocytes of the little skate *Raja erinacea* respond to osmotic stress with an outwardly rectifying anion conductance which is thought to be mediated by the volume-sensitive organic osmolyte/anion channel (VSOAC, Jackson, et al., *Am. J. Physiol.* 270: C57-C66, 1996). This anionic conductance has been shown to be inhibited by exposure to extracellular mercury salts (Ballatori and Boyer, *Toxicol. Appl. Pharmacol.* 140: 404, 1996). In this report we examined the effect of intra- and extracellular mercury on both basal and osmotically activated ion currents in this cell model.

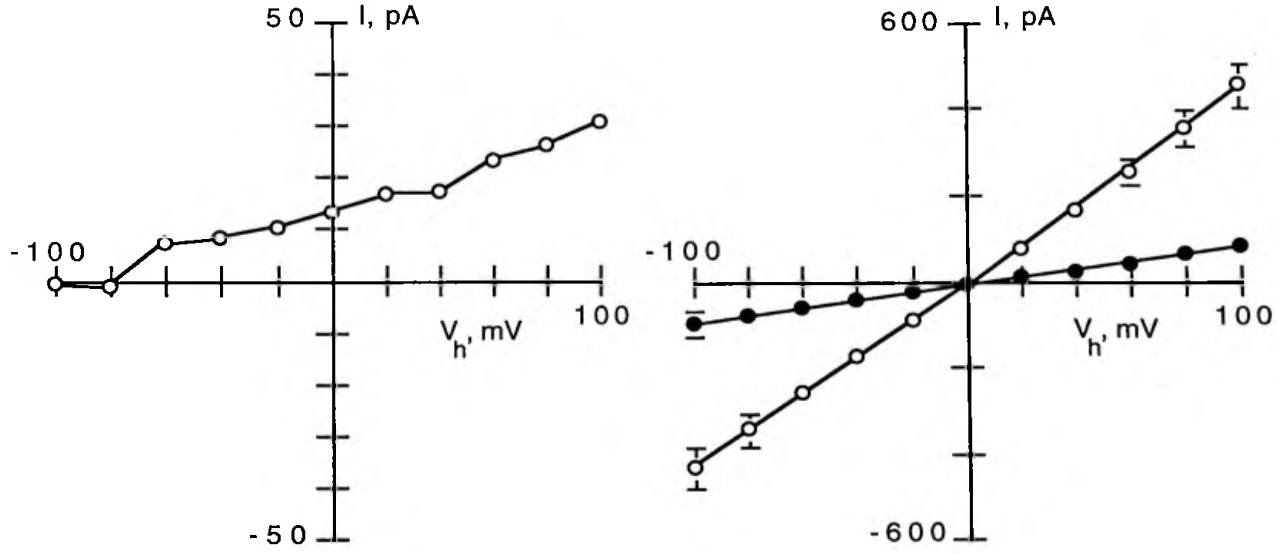

Fig. 1. Left: Hg-inhibited whole cell currents. Data are the delta between mean whole cell currents of control cells (n=6) and cells dialyzed with 10 μM intracellular HgCl2 (n=6). Right. Non-rectifying whole cell currents before (filled circles) and after (open circles) addition of 100 μM HgCl2 to the bathing solution (n=4).

Hepatocytes were isolated as previously described (Smith, et al., *J. Expt. Zool.* 241: 291-296, 1987), and allowed to settle on poly-L-lysine coated glass coverslips. The coverslips were then rinsed and placed in a bathing solution containing, in mM, 150

CsCl, 5 $MgSO_4$, 2.5 $CaCl_2$, 3 HEPES, 2 Tris, 320 sucrose, 350 urea, pH 7.4. Patch clamp experiments were carried out using micropipettes made from KG-33 glass capillaries (Garner Glass Co.) containing, in mM, 155 CsCl, 5 $MgSO_4$, 20 HEPES, 1 EGTA, 250 sucrose, 350 urea, 10 MgATP, 0.5 NaGTP, pH 7.2.

A basal current (0.16 nS/cell, n=6, data not shown) in control hepatocytes was inhibited by the presence of intracellular $HgCl_2$ (10 μM). The subtracted whole-cell conductance did not reverse between ±100 mV (Fig. 1, Left), indicating an electrodiffusional pathway enabling only cation currents. This is most consistent with Na+ pump-mediated ionic currents and is in agreement with previous reports showing an inhibition by mercury of Na+, K+-ATPase activity in the shark rectal gland (Pruett, et al., *Bull. MDIBL* 36: 17-18, 1997), and Na+ pump currents in the *Xenopus* oocyte (Smith, et al., *Bull. MDIBL* 34: 18-19, 1995). In contrast, basal currents increased 494% after addition of external 100 μM $HgCl_2$ (4.4 ± 0.5 nS/cell, n=4, vs. 0.89 ± 0.3, n=4, $p<0.01$, Fig. 1, Right). This electrodiffusional pathway was highly non-rectifying, thus suggesting a permeability to Cs and/or Cl. Whole cell currents of control hepatocytes were also increased after hypoosmotic shock with either external sucrose replacement or addition of water to the bathing solution. In this case, the osmotically activated currents displayed an outwardly rectifying Cl conductance (data not shown).

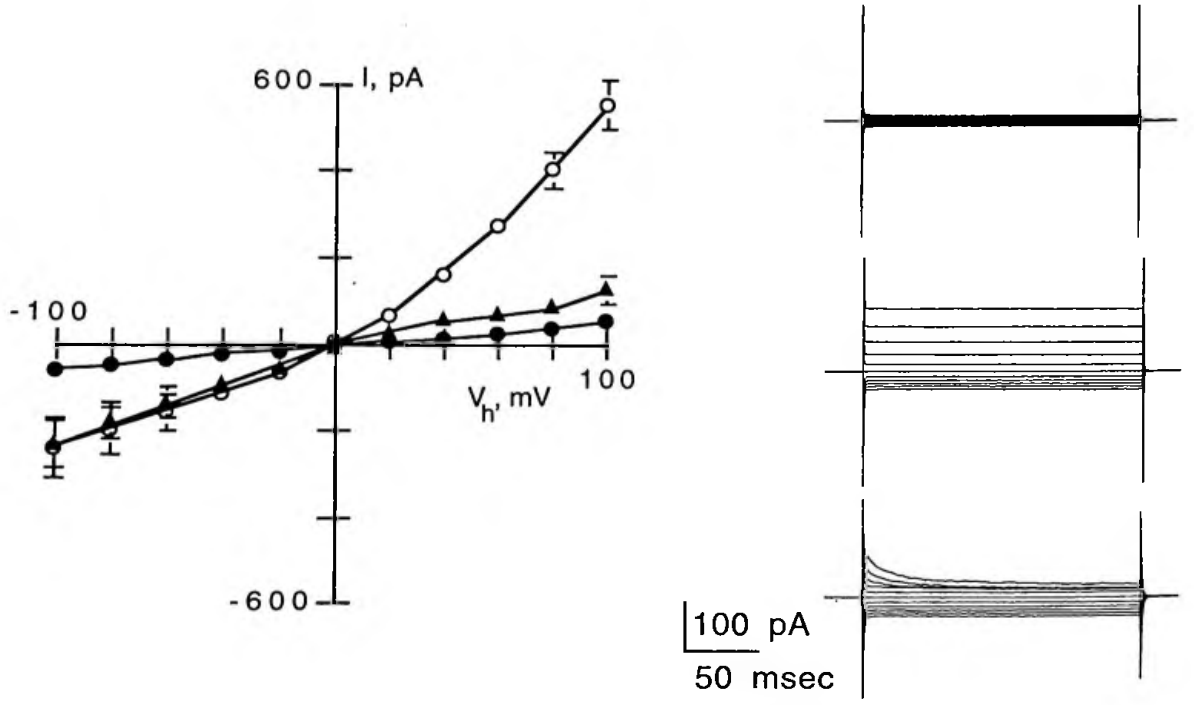

Fig. 2: Whole cell currents of skate hepatocytes with intracellular (10 μM) Hg. The figure shows basal currents (filled circles, top tracing, n=6), hypotonically stimulated (open circles, middle tracing, n=6), and DIDS (200 μM) inhibited currents (triangles, bottom tracing, n=4).

Exposure to osmotic shock of hepatocytes containing 10 μM $HgCl_2$ (dialysed through the patch pipette) also induced an outwardly rectifying Cl conductance (γ+=

6.6 ± 0.67 nS/cell, γ-= 2.1 ± 0.6 nS/cell, n=6, Fig. 2) However, only the currents seen at positive holding potentials were inhibited by external DIDS (200 μM, 2.3 ± 1.1 nS/cell, n=4, $p<0.05$). This is consistent with either a strongly voltage-dependent effect of DIDS, or with the possibility that more than one conductance is activated by osmotic shock.

The results in this report suggest at least two distinct effects of $HgCl_2$ on the ion conductances of skate hepatocytes, namely an inhibiton of the Na^+ pump by intracellular $HgCl_2$, and activation of an electrodiffusional cation pathway by extracellular $HgCl_2$. The data are also in agreement with an effect of intracellular $HgCl_2$ in the osmotically activated anion conductance of skate hepatocytes, as the currents in the presence of $HgCl_2$ strongly rectified. Further studies will be required to determine the nature of this latter transport mechanism.

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