EXTRACELLULAR $HgCl_2$ ACTIVATES AN ANION CONDUCTANCE IN ISOLATED HEPATOCYTES OF THE LITTLE SKATE, *RAJA ERINACEA*

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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). The VSOAC has been shown to be inhibited by exposure to extracellular mercury salts (Ballatori and Boyer, *Toxicol. Appl. Pharmacol.* 140: 404, 1996). Recent studies from our laboratory have also shown that extracellular mercury activates a linear conductance in these cells (Jackson, et al., *Bull. MDIBL* 37: 87-89, 1998). However, the exact nature of this conductance has yet to be determined.

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.

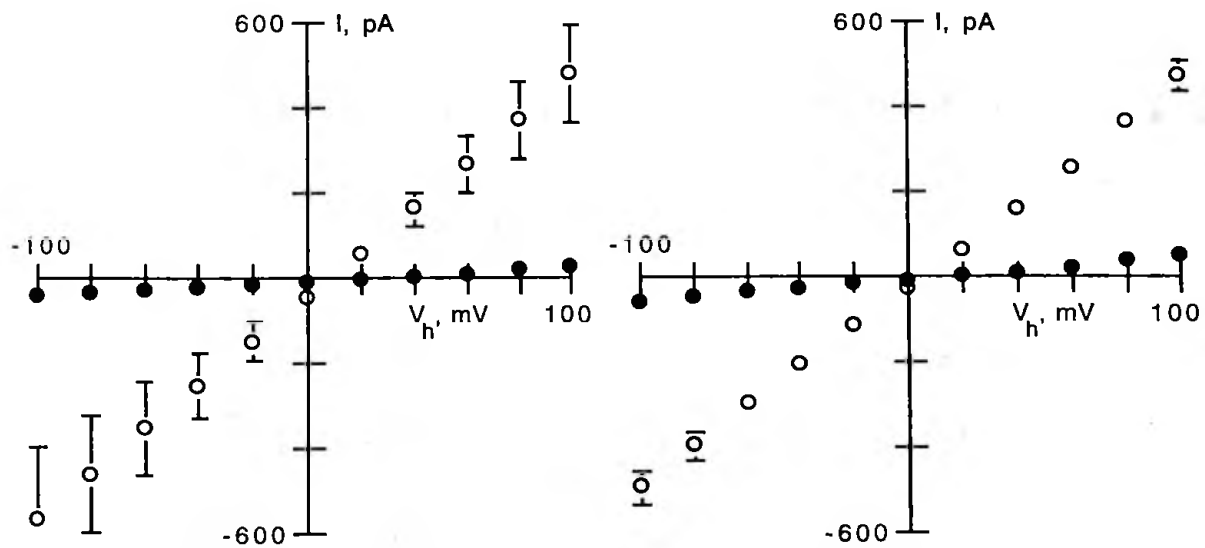

Figure 1. Whole cell currents activated by extracellular Hg in the absence (Left) or the presence (Right) of 10 μM intracellular HgCl2. Whole cell currents were obtained before (filled circles) and after (open circles) addition of 100 μM extracellular HgCl2. Figures are the mean ± SEM for 3 experiments.

The presence of intracellular 10 μM $HgCl_2$ had little effect on the basal conductance (0.32 ± 0.10 nS/cell vs. 0.52 ± 0.25 nS/cell, n=3, NS, in the presence and absence of intracellular $HgCl_2$, respectively). However, addition of 100 μM $HgCl_2$ to the bathing solution induced a 12.7 fold increase in linear whole cell currents in the absence of intracellular $HgCl_2$ (5.16 ± 1.43 nS/cell, n=3, $p<0.05$, Fig. 1, Left). A similar effect was observed in the presence of 10 μM intracellular $HgCl_2$ (4.73 ± 0.39 nS/ cell, n=3, $p<0.05$, Fig. 1, Right). In addition, activation of whole cell currents with external $HgCl_2$ also induced a shift in the reversal potential (Er, 24.7 ± 7.35 mV vs. 4.36 ± 2.28 mV, n=4, $p<0.05$, for basal and Hg stimulated, respectively) consistent with an anion conductance.

To initiate a characterization of this conductance, extracellular CsCl was replaced with an equimolar concentration of N-methyl-D-glucamine Cl. In these experiments, extracellular $HgCl_2$ activated a linear whole cell conductance (5.80 ± 1.48 nS/cell vs. 0.43 ± 0.10 nS/cell, n=4, $p<0.02$, Fig. 2, Left). Perfusion with a solution containing NMG-Cl produced a slight decrease in the whole-cell conductance, but was not statistically different from values in symmetrical CsCl. This suggested that Hg^{2+} may activate an anion conductance. However, the Hg^{2+}-activated currents were insensitive to the Cl channel blocker DIDS (1 mM, Fig. 2, Left).

To further assess whether cation movement contributed to the Hg stimulated conductance, the cation channel blocker $BaCl_2$ was tested. Addition of 1 mM $BaCl_2$ to the bathing solution had no effect on the Hg^{2+}-induced whole cell currents (6.12 ± 0.58 nS/cell vs. 5.87 ± 0.32 nS/cell, n=4, NS, for before and after addition of $BaCl_2$, respectively, Fig. 2, Right).

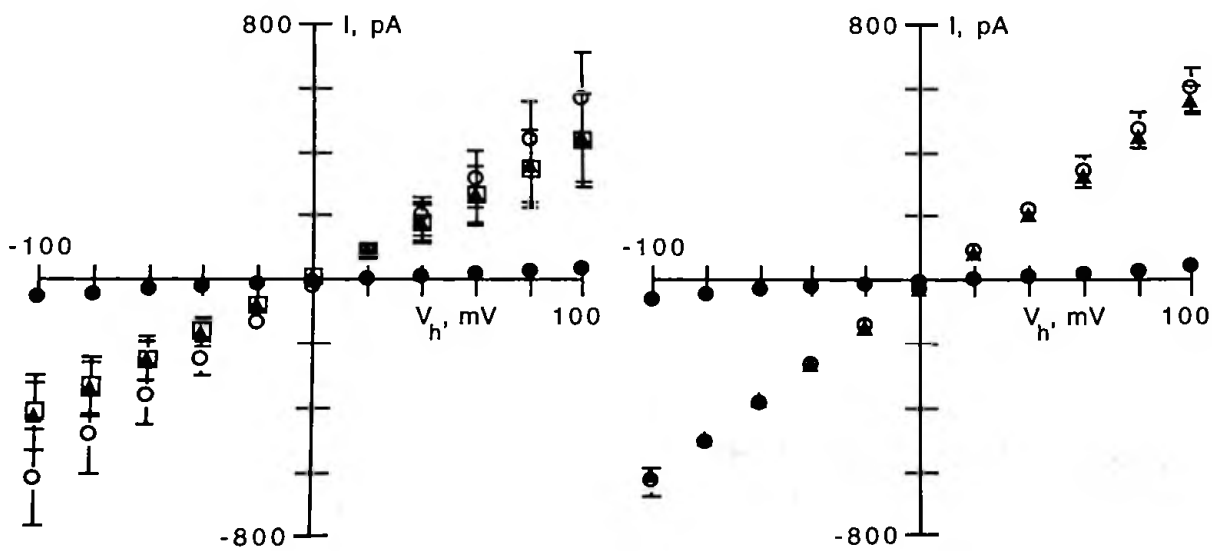

Figure 2. Left. Effect of cation replacement on the Hg activated whole cell currents. Addition of 100 μM extracellular HgCl2 (open circles) increased the basal whole cell currents (filled circles). A slight decrease in whole cell conductance was observed after bathing CsCl was replaced with NMG-Cl (triangles). The HgCl2 activated whole cell conductance was insensitive to 1 mM DIDS (squares). Right. Effect of Barium. HgCl2 activated whole cell currents (open circles) were insensitive to the cation channel blocker BaCl2 (1 mM, triangles). Basal currents are indicated by filled circles. Data are the mean ± SEM of 4 experiments.

The results in this report confirm our previous suggestion that intra- and extracellular Hg have different effects on the whole cell conductance of isolated skate hepatocytes (Jackson, et al., *Bull. MDIBL* 37: 87-89, 1998). Addition of intracellular Hg^{2+} (10 μM), a concentration which is sufficient to induce an effect on intracellular calcium in skate hepatocytes (Nathanson et al., *Cell Calcium*, 18:429-439, 1995) was largely without effect on the activation of the anion conductance described in this report. A small increase in basal whole cell currents was observed, however, (0.20 ± 0.01 nS/cell, n=4), which will require further investigation. Addition of external $HgCl_2$ was associated with the activation of an ion conductance not previously reported in skate hepatocytes. The characteristics of the external $HgCl_2$-induced whole cell conductance hepatocytes make it most likely associated with an anion permeability mediated by a pathway independent of the VSOAC. These characteristics include the linear whole cell conductance in symmetrical Cl^-, and the insensitivity to DIDS (for comparison, see Jackson, et al., *Am. J. Physiol.* 270: C57-C66, 1996). The distinct nature of these two conductances was further confirmed by the finding that in the late seasonal absence of a volume regulatory response in skate hepatocytes previously reported (Jackson, et al., *Am. J. Physiol.* 270: C57-C66, 1996), where little or no osmotically-activated rectifying Cl^- conductance was observed, addition of external $HgCl_2$ still activated a linear conductance (data not shown). While it has been previously reported that external Hg^{2+} induces a non-selective cation current (Ballatori & Boyer, *Toxicol. & App. Pharmacol.*, 140:404-410, 1996), the data in this report suggest that the Hg^{2+}-activated whole cell conductance would be equally permeable to Cs and NMG. In addition, the shift in Er induced by the $HgCl_2$-activated conductance was similar to that previously reported for the anion channel CFTR (Reisin, et al., *J. Biol. Chem.* 269: 20854-20591, 1994). Thus, the findings in this report are also consistent with the activation of an anion conductance.

CFTR function is characterized by a DIDS-insensitive and cAMP-activated linear whole cell conductance. It is therefore possible that "CFTR-like" transport mechanisms may be present in skate hepatocytes. Members of the ABC superfamily of transport proteins have been recently reported in hepatocytes, (Erlinger, *J. Gastroenterol. Hepatol.*, 11:575-579, 1996; Muller et al., *J Hepatol.*, 24:100-8, 1996; Elferink et al., *FASEB J.* 11:19-28, 1997; Roelofsen et al., *J. Cell Sci.* 111:1137-1145, 1998). It will thus be necessary to determine the functional presence of transport proteins similar in function to CFTR in the skate hepatocyte, and to further characterize the an anion component of the Hg^{2+}-activated whole cell conductance in these cells.

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