ENHANCEMENT OF CHLORIDE CURRENTS IN CULTURED SHARK RECTAL GLAND CELLS BY INCUBATION WITH MERCURY

Qingen Ke[1], Yinke Yang[1], John N. Forrest, Jr.[2] and Yong-Fu Xiao[1]
[1]Cardiovascular Division, Department of Medicine, Beth Israel Deaconess Medical Center Harvard Medical School, Boston, MA 02215
[2]Department of Medicine, Yale University School of Medicine, New Haven, CT 06510

Mercury contamination in our environment may cause severe neuronal and renal intoxication if the ion enters our body. Previous experiments of mercury uptake showed that compared to the brain and liver, the kidneys accumulated the highest levels of mercury (Hussain et al., *J. Environ. Sci. Health* 34:645-660, 1999). Renal failure has been found in mercury intoxication (Bank et al., *J. Clin. Invest.* 46:495-704, 1967; Flamenbaum et al., *Am. J. Physiol.* 224:305-311, 1973). A recent study in human autopsy samples from idrija residents and mercury mine workers showed that the highest mercury concentrations were found in endocrine glands and kidney cortex (Falnoga et al., *Environ. Res.* 84:211-218, 2000). The rectal gland of the spiny dogfish, *Squalus acanthias*, participates in the regulation of blood homeostasis by secreting salt and water. The shark rectal gland (SRG) has been used as a useful model for studying electrolyte secretion due to its structural characteristics and experimental accessibility. The apical membrane of SRG cells is responsible for the secretion of salt by regulation of the movement of chloride ions through Cl^- channels (Greger et al., *Pflug. Arch.* 409:114-121, 1987). The shark rectal gland actively secrets slat and water in response to cAMP stimulation (Stoff et al., *J. Exp. Zool.* 199:433-448, 1977). Therefore, SRG Cl^- channels might be one of the important proteins affected by mercury exposure. The present study was designed to examine the effects of mercury incubation on Cl^- currents in primary cultured SRG cells.

Different isoforms of cytochrome P450 (P450) have been found in renal tissues. P450 activities and P450-mediated AA metabolites may play important roles in some diseases. For example, epoxyeicosatrienoic acids (EETs) in the kidney act as a key factor for the development of spontaneous or salt-induced hypertension in rats (Basu et al., *Hypertension* 24:480-485, 1994; Da Silva et al., *Am. J. Med. Sci.* 307:173-181, 1994). In addition, urinary excretion of epoxygenase metabolites is increased during pregnancy-induced hypertension in humans (Catella et al., *PNAS USA* 87:5893-5897, 1990). Therefore, P450 may play an important role in renal function. This study investigated whether subchronic exposure to mercury affected Cl^- currents and P450 expression in cultured SRG cells.

Single cells enzymatically isolated from SRG were plated on glass coverslips or in culture dishes. Cells were cultured for 6 to 10 days until they were used for experiments. The whole-cell Cl^- current (I_{Cl}) was recorded by the whole-cell voltage-clamp method. The pipette solution contained (in mM) 240 CsCl or KCl, 20 Hepes, 0.2 EGTA, 5 MgATP, 1 $MgCl_2$, 70 TMAO, 300 urea, pH 7.5. The bath solution contained (in mM) 270 NaCl, 250 urea, 5 CsCl, 5 $CaCl_2$, 20 Hepes, 3 $MgCl_2$, 1 glucose, pH 7.5. Compounds used in this study were obtained from Sigma (St. Louis, MO). Application of compounds to patch-clamped SRG cells was via a fast perfusion system. Subchronic application of $HgCl_2$ was by

incubation of primary cultured SRG cells with mercury for 48 hrs. In washout experiments SRG cells incubated with mercury for 48 hrs were washed 3 times with a mercury-free solution during another 48 hrs. Patch-clamp experiments in $HgCl_2$-incubated SRG cells started after 5-min washout with the mercury-free bath solution. Experiments were carried out at 21-23^{0}C. Voltage-dependent currents were elicited by 400-ms pulses from -100 to 100 mV in 20-mV increments every 5 s. The amplitude of currents was measured at 200-ms place of each current trace. The membrane holding potential was 0 mV. I_{Cl} recorded immediately after rupture of the membrane was referred as control. The values of whole-cell Cl^- conductance were calculated from current-voltage relationship recorded after 0 min and 15 min dialysis of phosphatase I & II. Difference, $p < 0.05$, was considered as statistical significance tested by the Student's *t*-test.

Cl^- currents were enhanced in SRG cells incubated with 10 μM $HgCl_2$ for 48 hrs. This increase in I_{Cl} was reversed by washout of mercury for three times during another 48 hrs. In addition, intracellular dialysis with 20 units of phosphatase I plus 10 units phosphatase II for 15 min to dephosphorylate the channel protein significantly attenuated the $HgCl_2$-incubated increase in I_{Cl}. The whole-cell Cl^- conductance was attenuated from 6.97 ± 0.99 nS for the initial to 1.87 ± 0.42 nS for 15 min dialysis (n = 6, $p < 0.005$), respectively. In contrast, intracellular dialysis with 10 μM $HgCl_2$ for 20 to 30 min did not alter the amplitude of I_{Cl}. Western blot analysis shows that the protein level of cystic fibrosis transmembrane conductance regulator (CFTR) was not altered in SRG cells incubated with 10 μM $HgCl_2$ for 48 hrs.

Our previous data showed that 11,12-EET enhanced I_{Cl} in cultured SRG cells. To determine whether the increase in I_{Cl} caused by mercury incubation resulted from an induction of P450 expression in SRG cells, we carried out a group of experiments by addition of a P450 inhibitor. Coincubation with the P450 inhibitor clotrimazole (10 μM) abolished the increase in I_{Cl} in mercury-incubated SRG cells. The whole-cell Cl^- conductance was attenuated from 8.39 ± 1.14 nS for $HgCl_2$-treated SRG cells (n = 12) to 2.13 ± 0.70 nS for $HgCl_2$- plus clot-treated cells (n = 7, $p < 0.001$), respectively. Figure 1 shows that mercury incubation of SRG cells for 48 hrs produced an induction of the P450 CYP1A1, but not CYP2J2 (data not shown). The induction is more profound for 10 μM than for 1 μM mercury in the culture medium.

The present results demonstrate that incubation with $HgCl_2$ for 48 hrs enhanced Cl^- currents in cultured SRG cells. However, direct intracellular dialysis or 5 min incubation with mercury did not alter I_{Cl}. The difference may result from longer or shorter period of mercury exposure. The enhancement of I_{Cl} in SRG cells after incubation with mercury for 48 hrs is probably due to an intracellular mercury accumulation, which alters intracellular signaling process. The mercury-induced increase in I_{Cl} seems not due to a direct effect on Cl^- channels, because intracellular dialysis with mercury did not alter I_{Cl}. Therefore, the intracellular effects of mercury on I_{Cl} require longer exposure time. It is known that activation of Cl^- channels in SRG cells requires cAMP-dependent phosphorylation of the channel protein (Stoff et al., *J. Exp. Zool.* 199:433-448, 1977); Devor et al., *Am. J. Physiol.* 268:C70-79, 1995). Thus, it is possible that the mercury-incubated increase in I_{Cl} is a sequela of enhanced phosphorylation of Cl^- channels, which takes time to affect channel

function. This hypothesis is supported by the results that dephosphorylation of the channel abolished the mercury-incubated increase in I_{Cl} and that intracellular dialysis or 5 min incubation with mercury did not alter I_{Cl}.

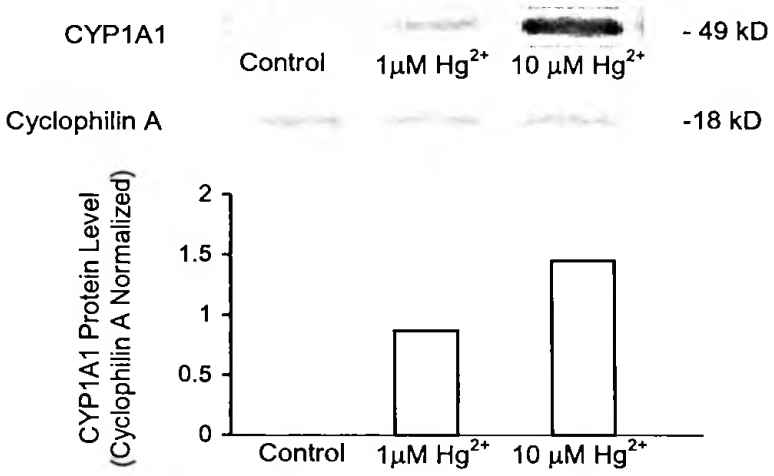

Figure 1. Induction of the cytochrome P450 CYP1A1 in cultured SRG cells incubated with mercury. Western blot analysis was carried out in cultured SRG cells incubated with 1 or 10 μM $HgCl_2$ for 48 hrs. The protein levels of CYP1A1 were normalized by internal control of cyclophilin A in the absence or presence of mercury.

The mercury-incubated increase in I_{Cl} may relate to an enhancement of Cl^- channel phosphorylation by an induction of important proteins, including P450 enzymes, which is able to stimulate second message systems. The mechanism of the mercury-incubated increase in I_{Cl} needs to be elucidated. In addition, molecular and cellular bases of P450 modulation of Cl^- channels are important for assessment of the role of P450 in mercury toxicity and in renal pathophysiology. Mercury-elicited oxidative stress-like phenomenon is one proposed mechanism of mercury toxicity. P450-mediated reactions require molecular oxygen and NADPH which plays an important role in cellular toxicity of free radicals. Activation of Cl^- channels in SRG cells requires cAMP-dependent phosphorylation of the channel protein. Therefore, dephosphorylation of the channel protein resulted in an attenuation of the mercury-incubated increase in I_{Cl}. In the present study, an induction of CYP1A1, not CYP2J2, was found in mercury-incubated SRG cells. More experiments are required to determine whether mercury-incubation alters protein levels of other P450 isoforms. The significance of an induction of CYP1A1 need to be elucidated in mercury-incubated SRG cells.

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