OPPOSITE EFFECTS OF MERCURY ON Cl^- CURRENTS IN ACUTELY AND SUBCHRONICALLY MERCURY-TREATED SHARK RECTAL GLAND CELLS

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Mercury, a major environmental contaminant, causes a variety of toxic effects on different organs when this material enters biological organisms including man. The kidney is one of the main target organs for mercury toxicity. Renal failure has been found in mercury intoxication (Bank et al., *J. Clin. Invest.* 46:695-704, 1967; Flamenbaum et al., *Am. J. Physiol.* 224:305-311, 1973). Shark rectal gland (SRG) participates in the regulation of the blood volume by secreting salt and water. The movement of chloride ions through cAMP-activated Cl^- channels in the apical membrane of rectal gland cells is believed to be responsible for the secretion of NaCl in the spiny dogfish, *Squalus acanthias*. Therefore, SRG Cl^- channels may be one of the important proteins affected by mercury exposure. The present study examined the effects of acute and subchronic exposure to mercury on Cl^- currents in primary cultured SRG cells.

The methods used for isolation and culture of single SRG cells were similar to those described previously (Valentich & Forrest, Am. J. Physiol. 260:C813-C823, 1991). Cells plated on glass coverslips for 6 to 10 days in culture were used for whole-cell voltage clamp experiments (Xiao, Life Sci. 60:2231-2243, 1997). The pipette solution contained (in mM) 240 CsCl or KCl, 20 Hepes, 0.2 EGTA, 5 MgATP, 1 $MgCl_2$, 70 TMAO, 300 urea, and pH 7.2. The bath solution contained (in mM) 270 NaCl, 250 urea, 5 CsCl, 5 $CaCl_2$, 20 Hepes, 3 $MgCl_2$, 1 glucose, and pH 7.2. Compounds used in this study were obtained from Sigma (St. Louis, MO). Acute application of $HgCl_2$ to patch-clamped SRG cells was via a fast perfusion system. Subchronic application of $HgCl_2$ was by incubation of primary cultured 5-days-old SRG cells with $HgCl_2$ for 48 hrs. Patch-clamp experiments in the $HgCl_2$-incubated SRG cells were carried out after 5-min washout of $HgCl_2$ culture medium with the mercury-free bath solution. Experiments were conducted at 21-23^{0}C. The amplitude of currents was measured at 200-ms place of each current trace. Statistical difference was examined by the Student's *t*-test and $p < 0.05$ was considered significantly different.

First, we looked at the acute effects of mercury on the whole-cell Cl^- current (I_{Cl}) in cultured SRG cells. Bath perfusion of a cocktail solution containing 10 μM forskolin (F), 150 μM 1-isobutyl-3-methylxanthine (IBMX, I), and 500 μM 8-bromoadenosine monophosphate (cAMP, C) markedly enhanced I_{Cl} (Fig. 1A, F-I-C). Addition of 10 μM $HgCl_2$ inhibited the cAMP-activated Cl^- currents (Fig. 1A, F-I-C & $HgCl_2$). The average current at −100 mV was increased by 7-fold, from −90 ± 18 pA to −621 ± 160 pA (mean ± SEM, $n = 11$, $p < 0.01$). The current was returned to −170 ± 21 pA after 8-min washout of the cocktail solution. After 10 μM $HgCl_2$ was added to the cocktail solution, the cAMP-activated I_{Cl} was significantly inhibited, from −621 ± 160 pA for F-I-C to -245 ± 27 pA for F-I-C & $HgCl_2$ ($n = 11$, $p < 0.01$, Fig. 1B). Fig. 1C shows the whole-cell Cl^-

conductance in the absence (Control) and presence (F-I-C, F-I-C & $HgCl_2$) of cocktail stimulators. The Cl^- conductance was increased about 4.4 fold after cAMP stimulation ($p < 0.01$, $n = 11$) and significantly inhibited by 10 μM $HgCl_2$ ($p < 0.05$). The difference between control and F-I-C & $HgCl_2$ was not significant ($p > 0.05$).

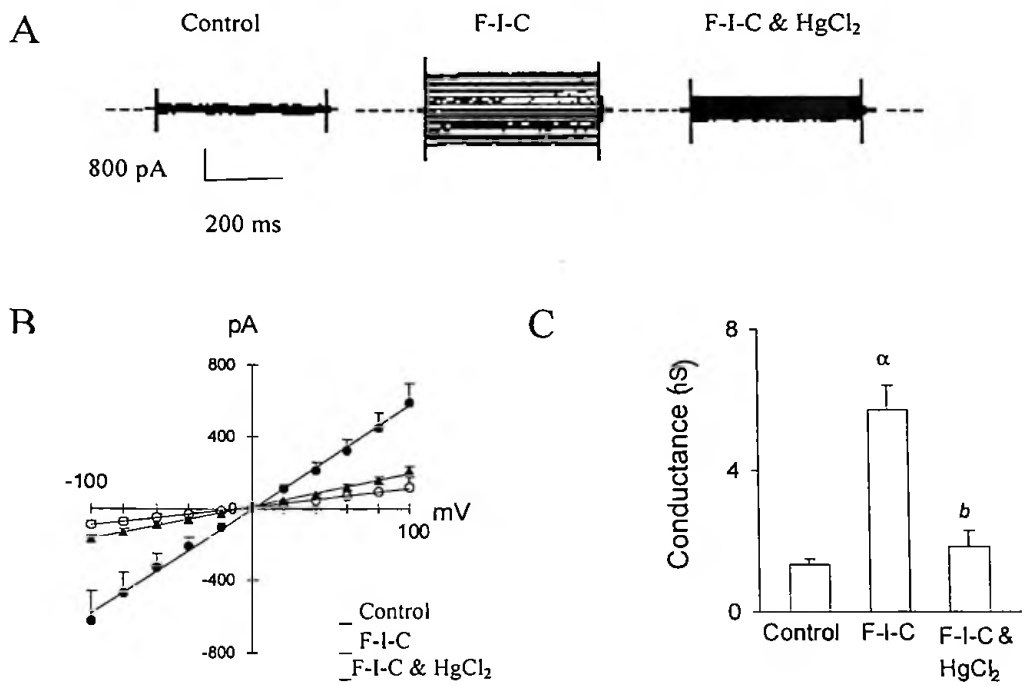

Figure 1. Acute effect of mercury on Cl^- currents in primary cultured SRG cells. **A.** Voltage-dependent current traces were elicited in the absence (Control) and presence of cocktail (F-I-C), and cocktail plus 10 μM $HgCl_2$ (F-I-C & $HgCl_2$). The membrane potential was held at 0 mV and currents were activated by 400-ms pulses from -100 to 100 mV in 20-mV increments every 5 s. F-I-C is the abbreviation of the cocktail: 10 μM forskolin (F), 150 μM IBMX (I), and 500 μM 8-bromo-cAMP (C). The dashed horizontal line represents the zero current level. **B.** The current-voltage relationship curves of I_{Cl} ($n = 11$) are shown for control (_), cocktail stimulation (_), and cocktail plus $HgCl_2$ (_). **C.** The values of whole-cell Cl^- conductance were calculated from the current-voltage relationship in the absence (Control) and presence of cocktail (F-I-C), and cocktail plus 10 μM $HgCl_2$ (F-I-C & $HgCl_2$). *a*, $p < 0.01$, vs. control; *b*, $p < 0.05$, vs. F-I-C.

To test the effects of subchronic exposure of mercury on Cl^- currents, we incubated cultured 5-day-old SRG cells with 10 μM $HgCl_2$ for 48 hrs. We were surprised by the results of $HgCl_2$ on Cl^- currents in mercury-incubated SRG cells. Fig. 2 shows that the base Cl^- current was markedly increased in SRG cells incubated with 10 μM $HgCl_2$ for 48 hrs. The Cl^- conductance was increased from -1.33 ± 0.3 nS for control ($n = 11$) to -6.57 ± 1.6 nS for mercury-incubated cells ($p < 0.01$, $n = 12$), respectively. In addition, I_{Cl} was also enhanced by the stimulation of the cocktail solution in mercury-incubated SRG cells (Fig. 3). The current densities measured at -80 mV were -5.6 ± 0.9 pA/pF for control (Control), -23.2 ± 7.9 pA/pF for cocktail (F-I-C), -18.5 ± 4.2 pA/pF for

mercury incubation ($HgCl_2$), -36.3 ± 8.2 pA/pF for mercury incubation plus cocktail ($HgCl_2$ & F-I-C), respectively.

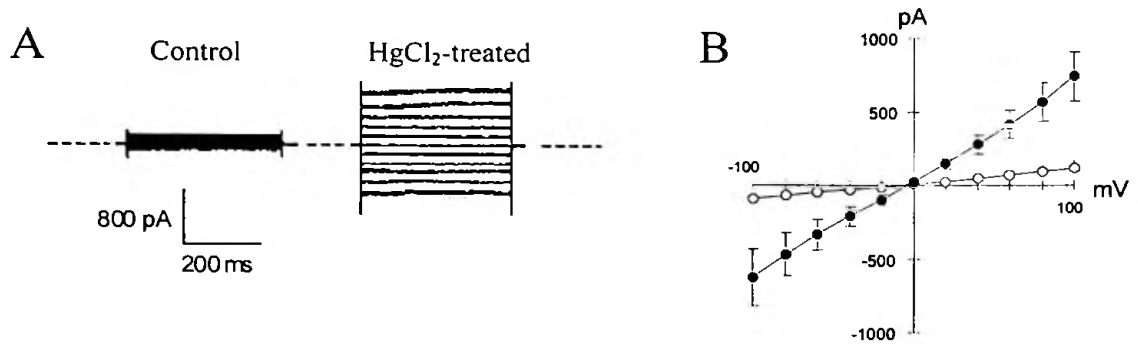

Figure 2. Enhancement of base Cl^- currents in mercury-incubated SRG cells. **A.** Voltage-dependent current traces were elicited in control (Control) and mercury-incubated (10 μM $HgCl_2$ for 48 hrs) SRG cells ($HgCl_2$-treated). The membrane potential was held at 0 mV and currents were activated by 400-ms pulses from -100 to 100 mV in 20-mV increments every 5 s. The dashed horizontal line represents the zero current level. **B.** The current-voltage relationship curves of I_{Cl} (n = 11) are shown for control (_, n = 11) and $HgCl_2$-treated SRG cells (_, n = 12).

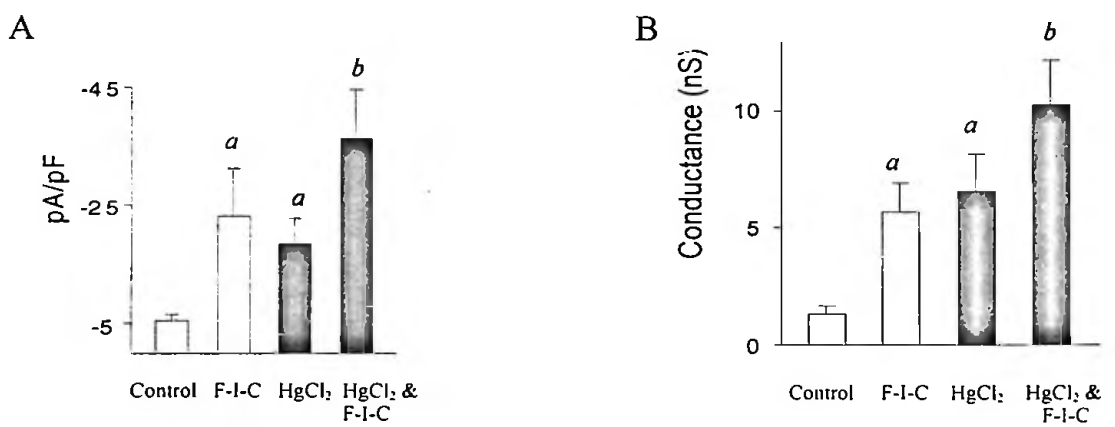

Figure 3. Cyclic AMP-activated Cl^- currents in control and mercury-incubated SRG cells. **A.** Current densities were calculated by dividing the amplitude of Cl^- currents by the cell membrane capacitance. Currents were activated by 400-ms pulses from a holding potential of 0 mV to –100 mV every 5 s. **B.** The values of whole-cell Cl^- conductance are shown for the untreated group (Control and F-I-C) and mercury-incubated group ($HgCl_2$ and $HgCl_2$ & F-I-C). *a*, $p < 0.01$, vs. control; *b*, $p < 0.05$, vs. $HgCl_2$ alone.

Our results show that the effects of mercury on Cl^- currents in cultured SRG cells are different when this compound was applied acutely and subchronically. The underlying mechanism of these different effects remains to be delineated. We speculate that the acute effect of mercury may result from a direct block of Cl^- channels and the subchronic effect may relate to its toxic actions on activities of second message systems or on expression of some important proteins, including the Cl^- channel itself.

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