ENDOCRINE DISRUPTION BY HEAVY METALS: INSULIN-LIKE EFFECTS OF MERCURIC CHLORIDE ON HEXOSE TRANSPORT AND PROTEIN SYNTHESIS IN SKATE (*RAJA ERINACEA*) HEPATOCYTES

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Mercury and other heavy metals are environmental pollutants that remain a major health concern. While a firm relationship between low level exposure to environmental pollutants and animal or human health effects remains to be established, there is a growing consensus that many diseases have an environmental component. Information regarding the endocrine-like effects of heavy metals is limited; however, acute exposure to heavy metals such as mercury, cadmium, and vanadium, at micromolar concentrations, induce insulin-like actions on cellular metabolism (May, J. M. and C. S. Contoreggi, *J. Biol. Chem.* 257:4362-4368, 1982; Shechter, Y. *Diabetes* 39:1-5, 1990; Ezaki, O., *J. Biol. Chem.* 264:16118-16122, 1989; Barnes et al., unpublished data). The mechanism(s) by which heavy metals elicit these hormone-like effects of heavy metals remains to be determined. Understanding the potential endocrine disruptive capacity of mercury has important health implications since a large population is chronically exposed to low Hg levels.

The objective of the present study was to determine whether long-term exposure to low levels of Hg caused insulin-like effects in hepatocyte metabolism. This objective was addressed using cells in suspension or cells plated on 24 well tissue culture plates. Cells were incubated in medium containing no effectors, increasing concentrations of mercury (1.0 nM to 0.2 mM), insulin (0.1 μM), insulin-like growth factor 1 (IGF-1) (10 ng/ml), or decreasing osmolality (5-40% dilution of Elasmobranch Ringers, ER). Suspension cultures were prepared as previously reported (Ballatori, N. and Boyer, J.L., *Toxicol. Appl. Pharmacol.* 140:404-410, 1996). Cells for adherent culture were repeatedly washed in sterile ER and brought up to final density in Dulbecco's Modified Eagle's Medium/F12 media supplemented with triamine N-oxide (100mM), urea (350mM), NaCl (90mM), $CaCl_2$ (1.5mM), $MgCl_2$ (2.0mM), taurine (0.1mM), betaine (0.1mM), and penicillin/streptomycin. Hexose transport was measured by determining uptake of either ^{3}H-2-deoxy-glucose or ^{3}H-1-D-glucose. Preliminary experiments showed that hexose uptake was linear for 4 minutes and plateaued by 10 minutes. Protein synthesis was measured by the incorporation of ^{3}H-phenylalanine into TCA insoluble material. Preliminary experiments showed that incorporation of radiolabelled amino acid into TCA insoluble material was linear for at least 4 hours for cell suspensions and for 12 hours in cells cultured on plastic. Incorporation was inhibitable by 20 μM cycloheximide. Changes in cell volume were characterized as previously described (Ballatori, N. and Boyer, J.L., *Toxicol. Appl. Pharmacol.* 140:404-410, 1996).

Cell Volume: Our previous data suggested a link between cell volume regulation and the effects of insulin and Hg on metabolism (Barnes et al., unpublished data). Hg and osmotically-induced cell swelling increase cell volume in *Raja* liver, however, it was unknown whether insulin would swell hepatocytes as reported in mammalian liver (Haussinger, D. et al., *Am. J. Physiol.* 267:E343-E355,1994). Hypotonic media increased intracellular water space (IWS) within 2-5 minutes (59% increase) while $HgCl_2$ and insulin increased IWS after 1-2 hours 25 ($P< 0.02$) and 11% ($P< 0.06$) respectively. Observations of increased IWS due to insulin stimulation were inconsistant due to the difficulty in distinguishing small differences between treatments using isotopic methods.

Glucose Uptake: Hepatic glucose uptake was phloretin (250μM) sensitive and increased by osmotically-induced cell swelling (Table 1). Glucose uptake initially increased as a function of the change in cell volume, however initial rates of uptake remained elevated after cells underwent osmoregulation. Hepatocytes responded to hypotonic media by increasing IWS followed by a regulatory volume decrease within 30 minutes however the effects on metabolism can be observed

several hours later. Neither insulin nor IGF-1 had an effect on hepatocyte glucose uptake (Data not shown). Hg-stimulated glucose uptake was consistantly seen in more than half the experiments however, small procedural differences between experiments necessitate further trials before a relationship between Hg and glucose uptake can be established. Interestingly, the time course of mercury's effect on glucose uptake is similar to the time course of cell volume changes induced by Hg.

Table 1. Initial rates of ^{3}H-glucose (3.0μCi/ml) uptake of by hepatocyte cell suspensions; effects of Hg and osmotically induced cell swelling. Cells were cultured with effectors for 3 and 2 hours respectively for panels A and B. Data are expressed as mean cpm/μg protein ± SEM (n=3). Values significantly (Student's t test) differing from controls are denoted by * (P<0.05).

Panel A		Panel B	
Control	7.03 ± 0.53	Control	14.33 ± 0.04
1.0 μM Hg	10.21 ± 0.33*	40% dilution of Media	19.06 ± 1.38*
10 μM Hg	10.20 ± 0.26*	250 μM Phloretin	6.53 ± 0.42*
50 μM Hg	9.28 ± 0.43*	Dilution + Phloretin	5.94 ± 0.52*

Protein Synthesis: Skate hepatocytes in suspension were not observed to consistently alter phenylalanine incorporation due to treatment with insulin, Hg or osmotically-induced cell swelling. Cell swelling stimulated incorporation at 30 minutes (3-fold increase P<0.05) but was inhibitory during longer incubations (1-6 hours). Hg treatment consistently resulted in numerically higher values than control values, however, no statistically significant differences were observed for Hg incubations from 1-20 hours. High concentrations of Hg (10-50μM) inhibited phenylalanine incorporation. Insulin caused a small but not significant stimulation in phenylalanine incorporation (<7%). In contrast, hepatocytes cultured on tissue culture plastic resulted in a responded to insulin (100nM), Hg (100nM) or osmotically-induced cell swelling (5-40% dilution of media) by increasing phenylalanine incorporation into protein. Table 2 shows two representative experiments in which Hg and cell swelling stimulated phenylalanine incorporation. In these and other experiments (data not shown), Insulin, Hg and osmotically-induced cell swelling stimulated phenylalanine incorporation 30-50%, 60-70% and 30-250% respectively.

Table 2. Incorporation of ^{3}H-phenylalanine (0.4μCi/ml) by skate hepatocytes in two separate experiments. Cells in both experiments were cultured on tissue culture plastic with effectors for 24 hours. Data are expressed as mean cpm/μg protein ± SEM (n=4). Values significantly (Student's t test) differing from controls are denoted by * (P<0.05).

Control	7.46 ± 0.61	Control	1.81 ± 0.11
0.01μM Hg	9.42 ± 1.04	5% Dilution of Media	2.01 ± 0.19
0.05μM Hg	10.53 ± 0.89*	10% Dilution of Media	2.21 ± 0.13*
0.1μM Hg	12.62 ± 1.23*	20% Dilution of Media	2.38 ± 0.10*
0.5μM Hg	11.03 ± 0.75*	100 nM Insulin	2.51 ± 0.13*
1.0μM Hg	9.37 ± 0.57		

Osmotically induced cell swelling of hepatocytes significantly alters glucose uptake and protein synthesis. Osmotically-induced cell swelling appears to activate a persistent cell signal since the metabolic results exhibit a longer time course than the changes in cell volume. That is, swelling and osmoregulation occur in a matter of minutes and the changes in biochemistry (glucose uptake, protein synthesis) are observed for at least two days. Insulin and Hg increase intracellular water space and alter glucose uptake and protein synthesis suggesting a common mechanism of action. This common mechanism may represent a target for Hg and other xenobiotics in the disruption of insulin action.

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