# EFFECT OF $Hg^{2+}$ ON CYTOSOLIC $Ca^{2+}$ IN HEPATOCYTES ISOLATED FROM THE LITTLE SKATE RAJA ERINACEA

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Introduction. $Hg^{2+}$ is an environmental pollutant that adversely affects a number of secretion-related functions in skate hepatocytes, including cell volume regulation, $Na^+$-alanine co-transport, and plasma membrane ion permeability. Cytosolic $Ca^{2+}$ ($Ca_i^{2+}$) regulates secretion in many other types of tissue, and our preliminary work suggests that ATP-induced $Ca_i^{2+}$ signals regulate bile secretion in skate liver as well. The purpose of this study was to examine whether $Hg^{2+}$ affects $Ca_i^{2+}$ in isolated skate hepatocytes, and if so, to define the responsible mechanisms. Hepatocytes were isolated by collagenase perfusion, then loaded with the $Ca^{2+}$-sensitive dye indo-1 and examined by ratio spectrofluorometry using a Perkin-Elmer LS-5B spectrometer. Results. At lower concentrations (100 nM-5 μM), $Hg^{2+}$ induced no detectable change in $Ca_i^{2+}$. At higher concentrations (10 μM-1 mM), $Hg^{2+}$ induced a dose-dependent, progressive increase in $Ca_i^{2+}$ (Figure 1). This $Ca_i^{2+}$ increase began within seconds after addition of $Hg^{2+}$ and occurred even in $Ca^{2+}$-free medium. Pre-treatment of hepatocytes with the membrane-impermeant $Hg^{2+}$ chelator glutathione (GSH, 5 mM) blocked the $Ca_i^{2+}$ increase induced by 50 μM $Hg^{2+}$, while addition of GSH 2 min after exposure to $Hg^{2+}$ slowed but did not prevent further increases in $Ca_i^{2+}$. As with GSH, pre-treatment with the membrane-permeant $Hg^{2+}$ chelator dithiothreitol (DTT, 500 μM) blocked $Hg^{2+}$-induced increases in $Ca_i^{2+}$. Unlike GSH, however, addition of DTT 2 min after 50 μM $Hg^{2+}$ significantly decreased $Ca_i^{2+}$, returning it to near-baseline levels. The $Ca^{2+}$-ATPase inhibitor thapsigargin (2 μM) caused a sustained increase in $Ca_i^{2+}$, and addition of $Hg^{2+}$ resulted in a further, progressive $Ca_i^{2+}$ increase. Stimulation of hepatocytes with a maximal concentration of ATP (100 μM) increased $Ca_i^{2+}$ as well, after which addition of $Hg^{2+}$ also resulted in a further, progressive $Ca_i^{2+}$ increase. Acute effects of $Hg^{2+}$ on toxicity were examined in two ways. First, $Hg^{2+}$ (100 μM) induced no increase in propidium iodide uptake over 4 min, relative to untreated (control) hepatocytes. Second, no morphological changes were detected by light microscopy in $Hg^{2+}$-treated hepatocytes over this same time period. Summary. Together, these findings suggest: (1) $Hg^{2+}$ increases $Ca_i^{2+}$ in skate hepatocytes, (2) $Hg^{2+}$ must enter the hepatocytes for this $Ca_i^{2+}$ increase to occur, and (3) this increase is mediated by release of $Ca^{2+}$ from endogenous stores that are distinct from the thapsigargin-sensitive, ATP-mobilizable $Ca^{2+}$ stores. Furthermore, this acute effect of $Hg^{2+}$ on $Ca_i^{2+}$ does not cause or result from acute toxicity to hepatocytes. Additional work will be needed to define the intracellular source from which $Hg^{2+}$ releases $Ca^{2+}$ into the cytosol, and to determine if previously described effects of $Hg^{2+}$ on hepatocyte metabolism are mediated by these $Hg^{2+}$-induced $Ca_i^{2+}$ signals. Acknowledgements. This work was supported by a Young Investigator Award (to MHN) from the Center for Membrane Toxicity Studies (P30 ES03828), a Fiterman Award for Basic Research (to MHN) and a Student Research Award (to KM) from the American Gastroenterological Association, a Liver Scholar Award from the American Liver Foundation (to MHN), and the Hepatocyte Isolation and Morphology Core Facilities of the Yale Liver Center (P30 DK34989).

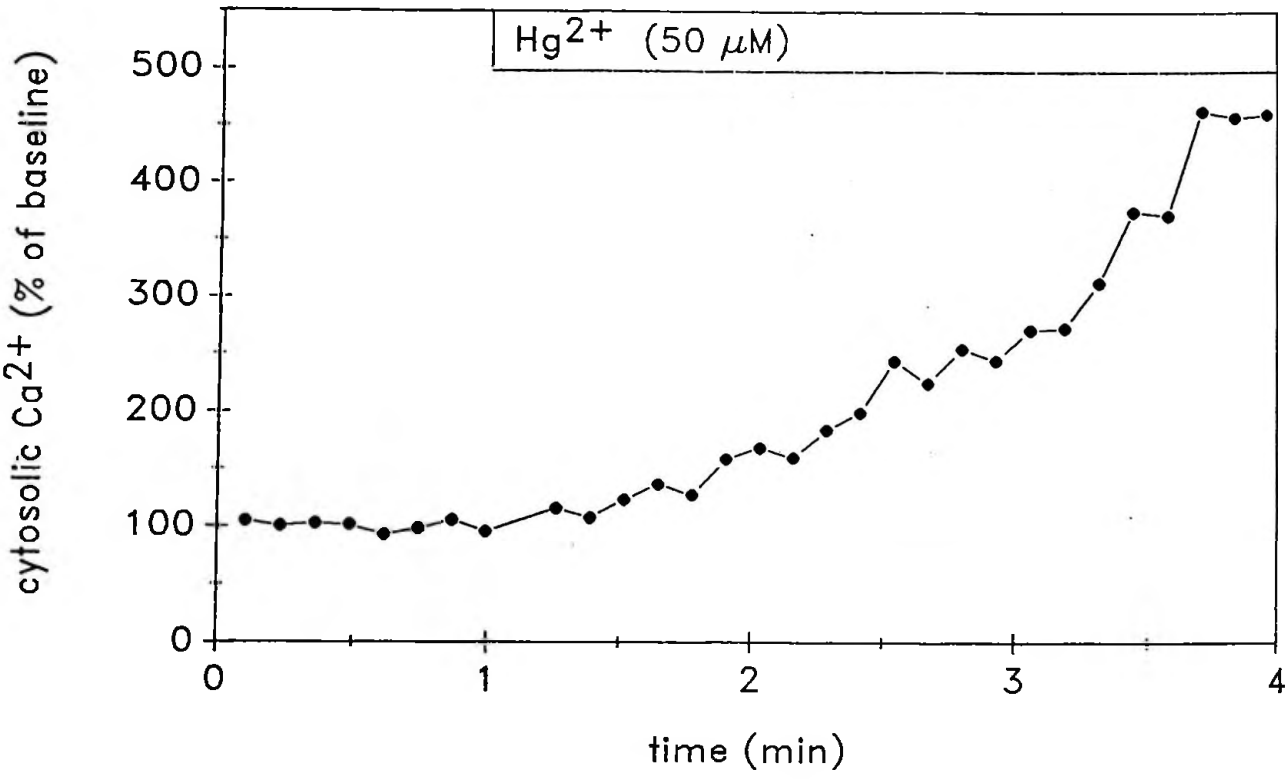


Figure 1. Effect of 50 μM $Hg^{2+}$ on $Ca_i^{2+}$ in isolated skate hepatocytes. $Hg^{2+}$ induces a progressive increase in $Ca_i^{2+}$ after a brief delay period. Pattern is typical of that seen in n=5 separate experiments.