EFFECT OF $HgCl_2$ AND $CdCl_2$ ON HEAT SHOCK PROTEIN EXPRESSION IN THE RECTAL GLAND OF SQUALUS ACANTHIAS

M. Joannidis[1], R. Solomon[2], P. Silva[3] and F.H. Epstein[1]
Beth Israel Hospital[1], New England Deaconess Hospital[3] and Joslin Diabetes Center[2],
Boston, MA 02215.
Mount Desert Island Biological Laboratory, Salsbury Cove, ME 04672.

Heat shock proteins (HSPs) were originally described as a group of proteins induced by exposure of cells to high temperatures (42°C) for a restricted time. Heat shock proteins are divided into four main families according to their size, HSP 20-30 kDa, ubiquitin (HSP 64), HSP 70, HSP 90 (Ananthan J. et al., Science 232: 522-524, 1986; Burdon R.H., Biochem. J. 240: 313-324, 1986). They are thought to be involved in protein folding and unfolding providing protection against denaturation and may play a role in degradation of denatured proteins and transport of newly synthesized polypeptides into mitochondria and the endoplasmic reticulum. Heat-treated cells become thermo-tolerant and resistant to other stressful conditions, including exposure to heavy metals and hypoxia, which also stimulate their production (Lindquist S., Ann. Rev. Biochem. 55: 1151-91, 1986; Currie R.W. et al., Circ. Res. 63: 543-549, 1988).

Because of marine pollution fish may be exposed to high doses of heavy metals raising the question of whether $HgCl_2$ or $CdCl_2$ might stimulate HSP in elasmobranch organs .

Isolated rectal glands of Squalus acanthias were perfused by the standard technique in our laboratory (Silva P. et al., Methods in Enzymology, 192: 754-66, 1990). Glands were stimulated by adding theophylline (0.25 mM) and cAMP (0.5 mM) to the perfusate. Unstimulated glands were perfused with $HgCl_2$ (10^{-6} and 10^{-5} M) or $CdCl_2$ (10^{-4} and 10^{-3} M). Glands were perfused for four hours and then snap frozen in liquid N_2. Total RNA was prepared by homogenization in guanidine isothiocyanate buffer and consecutive purification on $CsCl_2$ gradients. 10mg of total RNA were loaded onto a 1% agarose/formaldehyde gel, transferred to a nylon membrane and hybridized with cDNA probes for HSP 70 and HSP 64. Quantification was performed by scanning on PhosphoImager.

Stimulation by cAMP and theophylline over 4 hours did not change HSP expression. Furthermore no induction of either HSP64 or 70 could be found after 4h of perfusion with $HgCl_2$ at concentrations of 10^{-6} and 10^{-5} M. $CdCl_2$ induced a two fold increase in both HSP 64 and HSP 70 message at 10^{-4} M. At 10^{-3} M, $CdCl_2$ induced a five and four fold increase in mRNA for HSP 64 and 70, respectively. The higher concentrations of both heavy metals resulted in deterioration of rectal gland function over the 4h perfusion period characterized by reduced perfusion flow and chloride secretion.

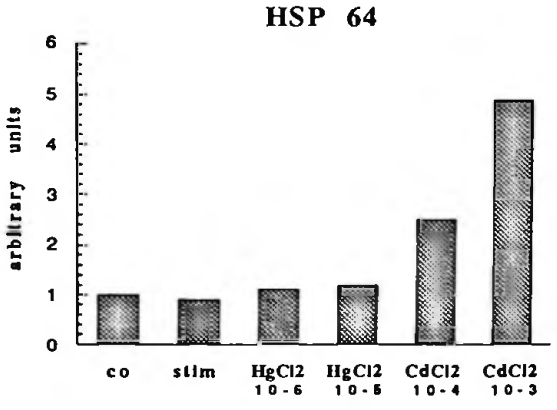

Figure 1. Induction of HSP 64 message by perfusion with standard media (co), during stimulation with cAMP+ theophylline (stim), with $HgCl_2$ (10^{-6}, 10^{-5} M) and $CdCl_2$ (10^{-4}, 10^{-3} M). Values are means of two RNA preparations.

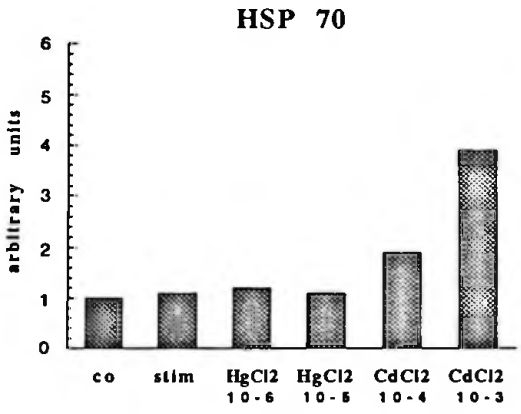

Figure 2. Induction of HSP 70 message by perfusion with standard media (co), during stimulation with cAMP+ theophylline (stim), with $HgCl_2$ (10^{-6}, 10^{-5} M) and $CdCl_2$ (10^{-4}, 10^{-3} M). Values are means of two RNA preparations.