Differential effects of permeant and non-permeant chelators of mercuric chloride on chloride secretion in the *in vitro* perfused rectal gland and cultured cell monolayers of the spiny dogfish shark (*Squalus acanthias*)

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Mercuric chloride inhibits chloride secretion in the shark rectal gland[4]. Mercury is known to alter protein action by binding to cysteinyl sulfhydryl (SH) groups and disrupting disulfide bonds. Previous work in this laboratory has suggested that the site of this action is the apical membrane protein CFTR[3,5]. We sought to examine the effects of two chelating agents, the cell permeant dithiothreitol (DTT), and the non-permeant glutathione (GSH), on mercuric chloride inhibition of stimulated chloride secretion. These chelators were tested in both the *in vitro* perfused shark rectal gland and in I_{sc} measurements of cultured monolayers of shark rectal gland cells.

Freshly excised rectal glands were perfused *in vitro* as previously described[2]. $HgCl_2$ and the chelating agent were perfused throughout the experiment. At t=30 min, the secretagogues forskolin (1 μM) and IBMX (100 μM) were added to the perfusate for the remainder of the experiment. Rectal gland tubular cells were cultured and grown on collagen coated nylon membranes, and Cl^- secretion was measured as I_{sc} in intact monolayers, as described previously[1].

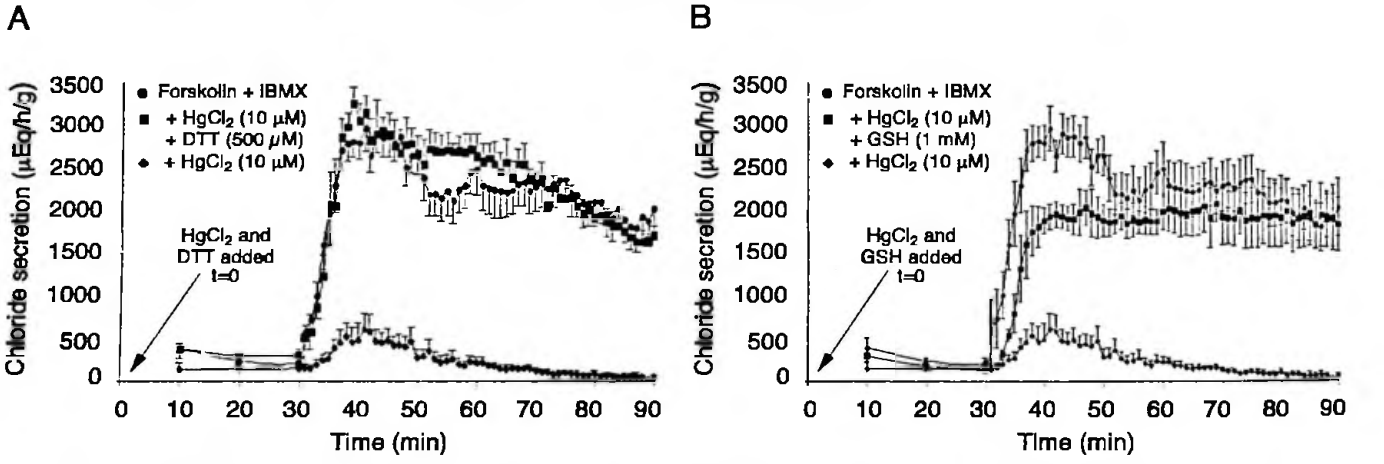

Figure 1. A) Prevention of the inhibitory effects of 10 μM $HgCl_2$ by 500 μM DTT. Both $HgCl_2$ and DTT were added to the perfusate at t=0 min (n=10 for control perfusions without mercury; n=4 with DTT and $HgCl_2$; n=5 with $HgCl_2$ alone). B) Prevention of the inhibitory effects of 10 μM $HgCl_2$ by 1 mM GSH. Both $HgCl_2$ and GSH were added to the perfusate at t=0 min (n=10 for control perfusions without mercury; n=3 with GSH and $HgCl_2$; n=5 with $HgCl_2$ alone. All values are mean ± SEM.

In the perfused gland, both DTT and GSH added concurrently with mercury significantly prevented $HgCl_2$ inhibition of chloride secretion. (Figure 1, Panels A and B). In I_{sc} measurements of cultured rectal gland cell monolayers, both DTT and GSH added prior to the addition of $HgCl_2$ also prevented inhibition (Figure 2, Panels A and C, Figure 3, Panel A).

When DTT was added to maximally inhibited cells, it reversed the inhibition by 53% (Figure 2, Panel B, Figure 3, Panel B). In contrast, the addition of GSH to maximally inhibited cells did not significantly reverse the inhibitory effects of inorganic mercury (Figure 2, Panel D; Figure 3, Panel B).

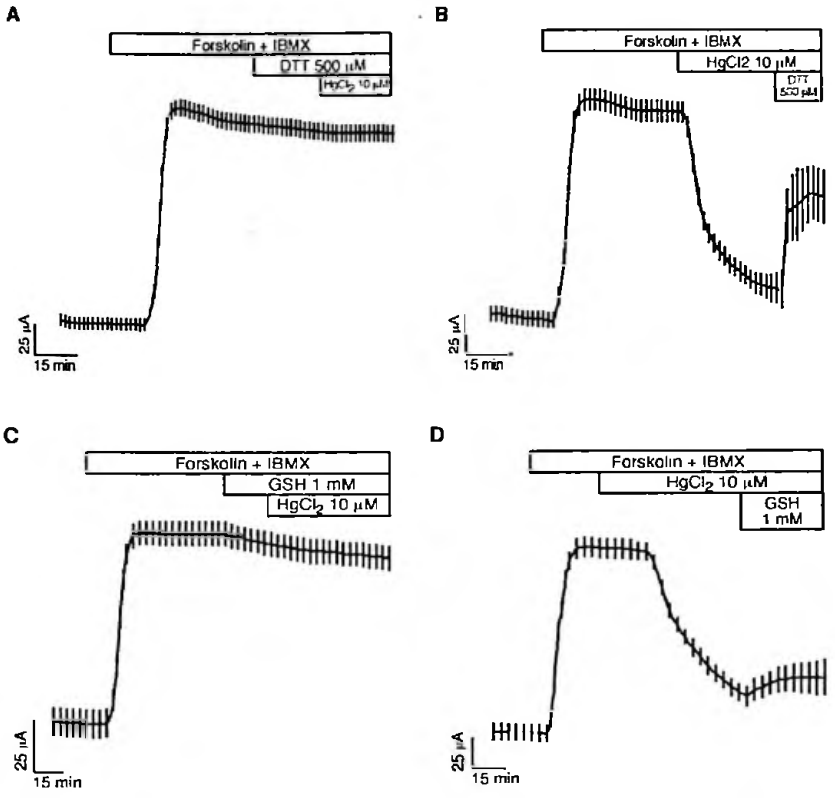

Figure 2. Representative I_{sc} tracings with DTT and GSH added to shark rectal gland monolayer cultures stimulated with forskolin (10 μM) and IBMX (100 μM) A) DTT (500 μM) added to the apical surface 17 min before the addition of $HgCl_2$ to the apical membrane prevents $HgCl_2$ inhibition. B) DTT (500 μM) added to the apical solution during maximum inhibition by inorganic mercury reverses the inhibition by 53%. C) GSH (1 mM) added to the apical surface approximately 17 min before the addition of $HgCl_2$ to the apical membrane prevents $HgCl_2$ inhibition. D) GSH (1 mM) added to the apical solution during maximum inhibition by inorganic mercury minimally reverses the inhibition.

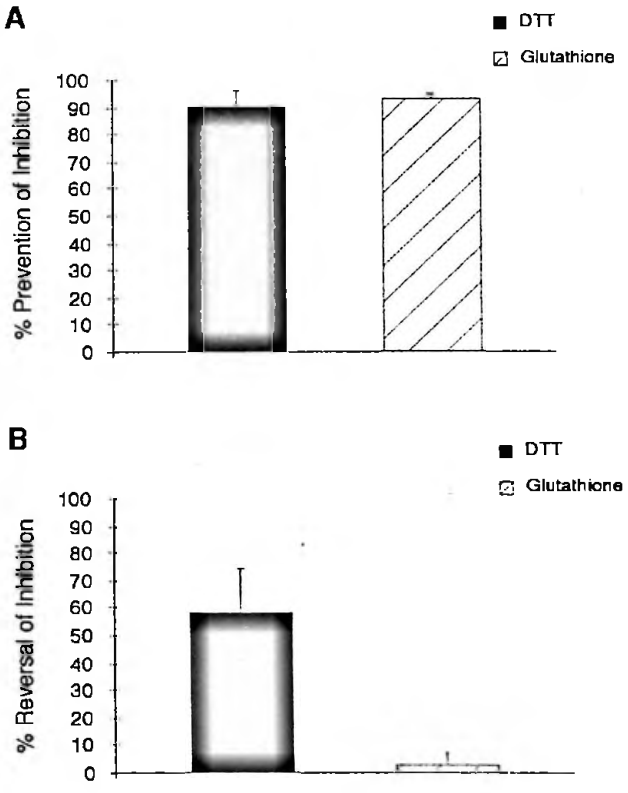

Figure 3. Mean percent prevention and reversal by DTT and GSH of the effects of mercury on I_{sc}. In all experiments shark rectal gland monolayer cultures were stimulated with forskolin (10 μM) and IBMX (100 μM). All values are mean ± SE. Both DTT and glutathione completely prevent the effects of mercury when added prior to the addition of $HgCl_2$ but only the membrane permeant chelator DTT significantly reverses these effects. A) Percent prevention of the effects of mercury by DTT (500 μM) and GSH (1 mM) added apically 17 min before apical $HgCl_2$ (10 μM) (n=4 for DTT; n= 9 for GSH). B) Percent reversal of the effects of $HgCl_2$ by DTT (200-500 μM) and GSH (1 mM) added apically during maximal inhibition by $HgCl_2$ (10 μM) ($p<0.0001$ for DTT, $p=0.34$ for GSH, n=10 for DTT; n= 8 for GSH). Percent prevention was calculated as $(100-(Isc_{FOR+IBMX+DTT} - Isc_{HgCl2}) / (Isc_{FOR+IBMX+DTT}) \times 100)$. Percent reversal was calculated as $[(Isc_{DTT} - Isc_{HgCl2}) / (Isc_{FOR+IBMX} - Isc_{HgCl2}) \times 100]$.

Both DTT and GSH, cell permeant and non-permeant chelators, respectively, completely prevented the inhibitory effects of mercury when added simultaneously with $HgCl_2$, indicating that both agents prevented mercury from reaching its site of action. In contrast, only the permeant chelator was able to reverse the effects of mercury when added under conditions of maximal inhibition. The ability of DTT, but not GSH, to reverse inhibition by $HgCl_2$ suggests that the site(s) at which inorganic mercury inhibits the CFTR chloride channel are intracellular cysteinyl residues.

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