POLARITY OF MERCURY TOXICITY IN THE SHARK (*SQUALUS ACANTHIAS*) RECTAL GLAND: APICAL CHLORIDE TRANSPORT AND SHARK CFTR CHANNELS EXPRESSED IN XENOPUS OOCYTES ARE HIGHLY SENSITIVE TO INORGANIC MERCURY

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Numerous membrane proteins in epithelial tissues have been proposed as sites for the toxic effects of mercury. In the perfused shark rectal gland, inorganic mercuric chloride (1-100 μM) inhibited chloride secretion in a dose dependent manner (Solomon et al., Bull. MDIBL 32:84-86, 1993) accompanied by an inhibition of rectal gland membrane Na-K-ATPase activity at (10-100 μM). Mercuric chloride also inhibited rectal gland membrane adenylate cyclase activity at high concentrations (Solomon et al., Bull. MDIBL 32:84-86, 1993).

The basolateral Na-K-2Cl co-transporter has also been suggested as a potential site for mercury toxicity (Kinne-Saffran and Kinne, Bull. MDIBL 33:93-94, 1994). Pruett et al. (Bull. MDIBL 36:17-18, 1997) determined the IC_{50} concentrations for mercury in rectal gland membranes as 1.4 μM for Na-K-ATPase activity and 20 μM for the Na-K-2Cl co-transporter. Based on rubidium uptake in tubular preparations, ^{125}I efflux in cultured rectal gland cells, and Na-K-ATPase assays in rectal gland membrane preparations, it was concluded that the inhibitory effect of mercury on chloride secretion was not on the efflux of potassium or chloride or Na-K-ATPase activity. The primary effect of mercury was considered to be inhibition of Na-K-2Cl co-transporter activity (Silva et al., Bull. MDIBL 34:51-52, 1995).

We sought to examine the polarity of the inhibitory effects of mercuric chloride on chloride secretion by employing primary culture monolayers of *Squalus acanthias* rectal gland tubular cells. This preparation exhibits high rates of epithelial transport, measured as short circuit current (I_{sc}), when stimulated by secretagogues and allows access to both basolateral and apical membranes in paired experiments (Valentich and Forrest, Amer. J. Physiol. 260:C813-23, 1991; Lehrich and Forrest, Amer. J. Physiol. 269:F594-600, 1995).

Figure 1 shows a representative experiment of paired rectal gland primary culture monolayers exposed to increasing doses of $HgCl_2$ following stimulation of I_{sc} by forskolin (10 μM) and IBMX (100 μM). Forskolin and IBMX increased I_{sc} from basal values of 36 ± 10.2 to 238 ± 56 μA/cm^2 (mean ± SEM, n=6). The addition of increasing concentrations of mercuric chloride showed far greater sensitivity when applied to the apical membrane solution (Figure 1, Panel A). Inhibition was always seen at 1 μM $HgCl_2$ when applied to the apical membrane whereas this dose did not have an effect when added to the basolateral membrane solution.

Figure 2 summarizes the mean percent inhibition of the forskolin/IBMX response by mercuric chloride in a series of paired experiments. $HgCl_2$ was applied to the apical versus basolateral membrane in monolayers of shark rectal gland tubular cells. Addition of low concentrations of mercuric chloride (1-10 μM) to the apical membrane solution resulted in greater inhibition of chloride transport compared to addition to the basolateral membrane solution.

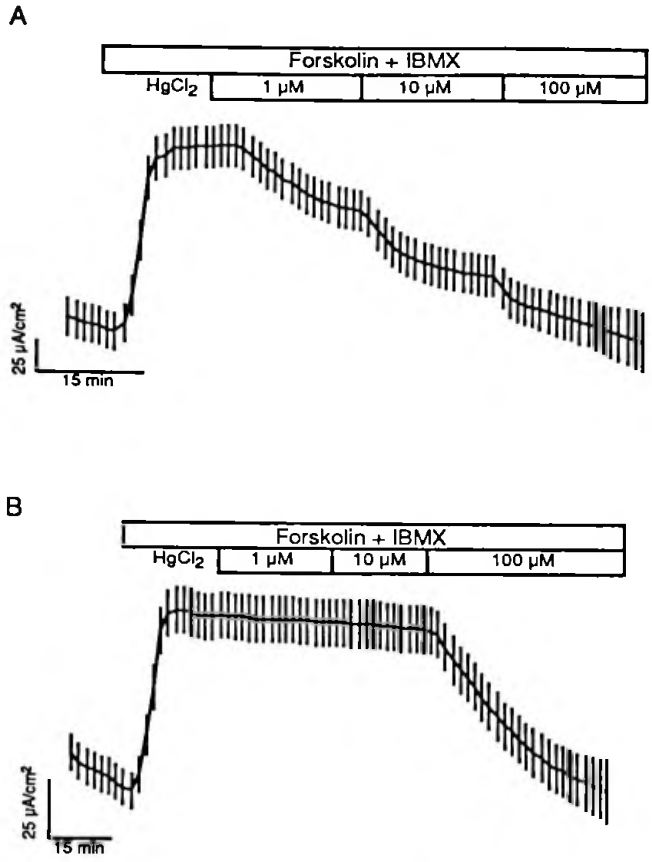

Figure 1. Representative Isc tracing of shark rectal gland cell monolayers. Panel A: apical membrane exposed to forskolin and IBMX with increasing concentrations of mercury. Panel B: basolateral membrane, same conditions.

We observed that the addition of dithiothreitol (DTT, 100 µM) and $HgCl_2$ (10 µM) simultaneously to the apical membrane entirely prevented the inhibitory effect of mercury. In the absence of DTT, apical $HgCl_2$ (10 µM) inhibited the IBMX/forskolin response by 50 ± 6% (n=4). Addition of DTT entirely prevented this inhibitory response (data not shown). DTT added after inhibition of I_{sc} by apically applied $HgCl_2$ partially but not entirely reversed the effects of inorganic mercury (data not shown). An organic mercuric compound, PCMSA which does not inhibit chloride transport in the perfused shark rectal gland, failed to inhibit chloride transport as measured by I_{sc}.

To examine the specific effect of mercuric chloride on shark CFTR chloride channels, we prepared cRNA of shark CFTR and detected membrane expression of the CFTR protein in CFTR injected oocytes by immunohistochemical techniques as previously described (Lehrich et al., J. Clin. Invest. 101:737-45, 1998). We then examined the effects of mercury on oocytes expressing shark CFTR. Addition of forskolin and IBMX (Figure 3) to the perfusate promptly stimulated an outward Cl^- conductance from 8 µS to 96 µS. When low concentrations of $HgCl_2$ (0.5-1.0 µM) were added to the oocyte perfusate, a prompt decline in chloride conductance was observed (Figure 3, representative experiment). In further preliminary studies, shark CFTR showed significantly greater sensitivity to mercury compared to human CFTR (data not shown; see abstract by Rizor et al., this volume).

Taken together the data indicate that in primary culture monolayers of rectal gland tubular cells mercuric chloride substantially inhibits chloride transport when applied to the apical vs basolateral membrane. The apical membrane is the site for chloride exit via CFTR and possibly other chloride channels, whereas the basolateral membrane has resident Na-K-ATPase, Na-K-2Cl co-transporters and potassium channels. Thus, in the primary culture monolayer system, CFTR

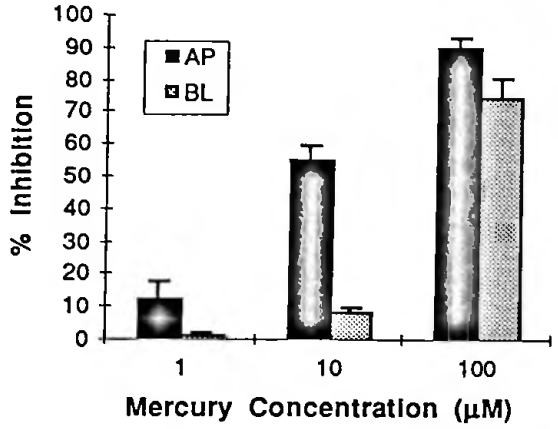

Figure 2. Percent inhibition of forskolin (10 µM) and IBMX (100 µM) of mercury chloride of apical (n=5) and basolaterial (n=5) membranes of shark rectal gland monolayers.

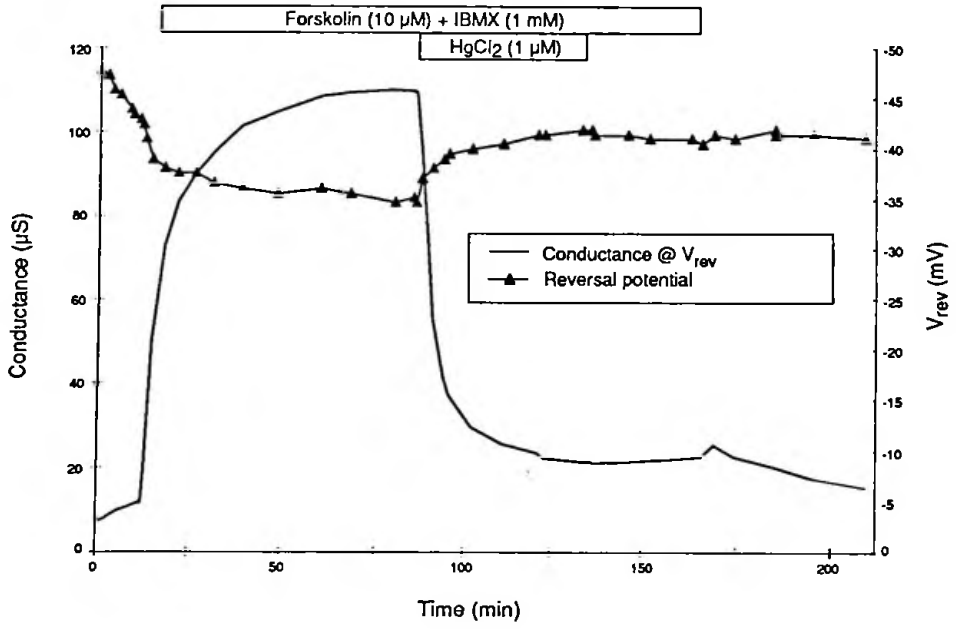

Figure 3. Cl^- conductance and reversal potential changes in an oocyte stimulated with forskolin and IBMX and then exposed to mercuric chloride (1 µM).

chloride channels may be the primary target for the toxic effects of mercury. Preliminary studies in oocytes expressing shark CFTR channels indicate high sensitivity of shark vs human CFTR chloride channels to mercuric chloride, consistent with differences in cysteine residues between the two channel proteins. Our data suggest that a major toxic effect of mercury in elasmobranch tissues and possibly other marine species may occur at CFTR chloride channels.

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