Disruption of Protein Metabolism in Squalus Acanthias Spermatocysts by Mercurials

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One consequence of heavy metal exposure is impaired reproduction (Clarkson et al, Reproductive and Developmental Toxicity of metals, Plenum Press, NY, 1983). Most studies in this area have focused on effects on the female reproductive tract, thus little information is available concerning the effects of heavy metals on spermatogenesis. Dogfish (Squalus acanthias) testis provides a unique model in which to study spermatogenesis. Unlike mammals, the dogfish testes are separated into distinct zones of germ cell maturation (Zones I, II, and III, or premeiotic, meiotic, and postmeiotic stages of spermatogenesis) and spermatocysts from each zone can be isolated and maintained in long-term culture (Callard et al. J. Exp. Zool. Suppl., 2:23, 1989). Described here are initial experiments in which we examined the effects of two mercurials (mercuric chloride, $HgCl_2$ and p-chloromercuriphenyl sulfonic acid, pCMBS), on ^{3}H-leucine labelling of the intracellular amino acid pool and protein in spermatocysts from the three zones.

Spermatocysts were isolated and cultured by the methods of Callard and Dubois (Bull. MDIBL 27:30, 1988). For experiments, cysts were incubated at 20°C and exposed to mercury for 24 hours; ^{3}H-leucine was added to the medium for last hour of exposure. Cysts were separated from the media by centrifugation, washed three times and treated with 10% trichloroacetic acid (TCA). Preliminary studies showed that TCA precipitable label was protein bound since 10 μM cycloheximide blocked >95% of the incorporation of label into this compartment. Label in the supernatant was derived from the intracellular amino acid pool and, as expected, was not affected by cycloheximide.

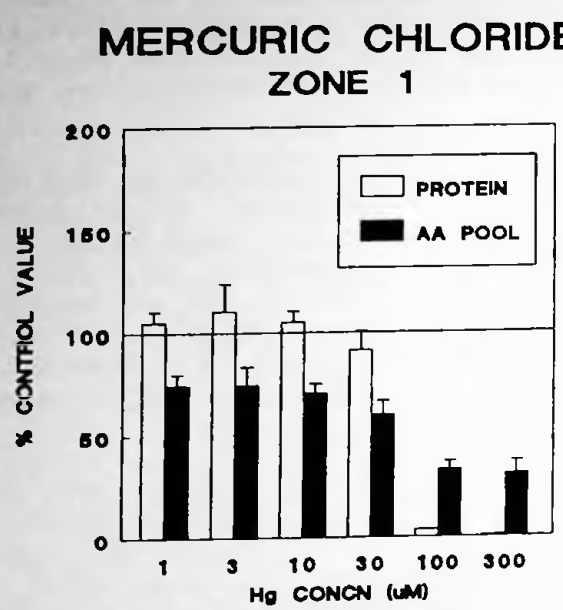
Figure 1 shows the results of 24 hours of $HgCl_2$ exposure on ^{3}H-leucine labelling of the two intracellular amino acid compartments. In spermatocysts from all three zones, mercury at 30 μM or below had no inhibitory effect on incorporation into protein, but between 30 and 100 μM, incorporation was essentially abolished. In Zone III spermatocysts, 1-30 μM $HgCl_2$ significantly stimulated incorporation ($P<0.05$ vs controls). A different pattern of effects was observed on labelling of the intracellular amino acid pool. Label in this pool was reduced significantly in Zone I after exposure to $HgCl_2$ concentrations as low as 1 μM; Zone II showed a significant decrease at 10 μM and greater; and Zone III was unaffected below 100 μM.

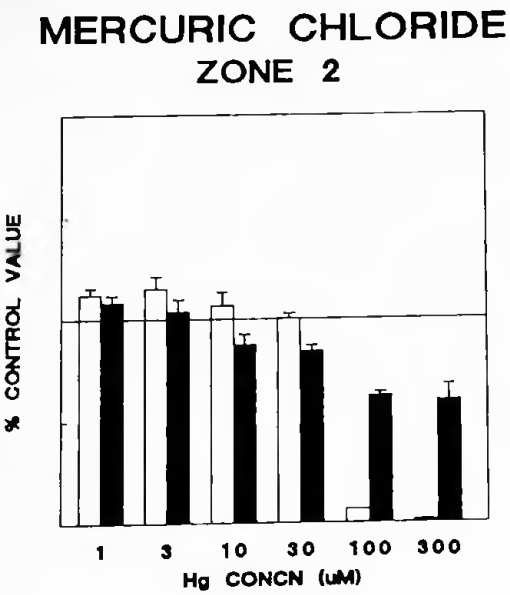
In similar experiments with pCMBS, a poorly permeable and less potent mercurial, we found that labeling of the intracellular amino acid pool and cell protein fell in parallel in all zones (Fig. 1). Thus, for pCMBS, the effects on protein synthesis could be explained primarily by a reduction in intracellular leucine label, presumably caused by inhibition of leucine uptake at the plasma membrane. In contrast, because a substantial fraction of $HgCl_2$ is not ionized in aqueous solutions, this mercurial is expected to rapidly penetrate cell membranes and act at both plasma membrane and intracellular sites. Consistent with intracellular actions Fig. 1 shows that in all three zones 100 μM $HgCl_2$ reduced leucine incorporation into protein to a much greater extent than labelling of the free amino acid pool. However, Fig. 1 also shows that in Zone III low

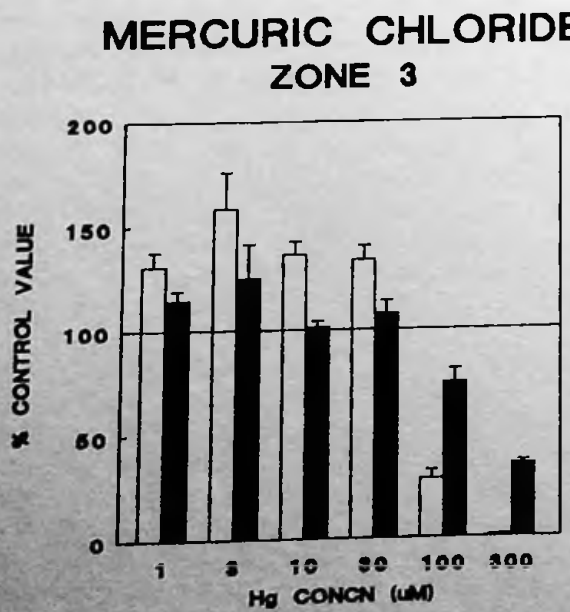
concentrations of $HgCl_2$ stimulate labelling of protein, but did not affect labeling of the amino acid pool. In Zone I, those same low concentrations reduced labeling of the amino acid pool but had no effect on the labeling of protein. One explanation for these findings is that $HgCl_2$ had a biphasic effect on absolute rates of protein synthesis in Zones I and III not occurring in Zone II. This biphasic effect was manifest by stimulation at low concentration and inhibition at higher concentrations.

Together, the present data indicate that protein metabolism in dogfish spermatocysts is disrupted by $HgCl_2$ and that some of the effects may be stage specific. Spermatocysts contain two interacting cell types, germ cells and sertoli cells. During spermatogenesis, changes take place not only in the relative proportion of the two cells within the cyst, but also the biochemical processes occurring within each. Thus it is important that we understand at each stage of spermatogenesis how toxins act in terms of their effects on each cell type and on the interaction between cells. This is a goal of future studies using the dogfish model.

FIGURE 1. Effects of mercurials on labeling of the intracellular free amino acid (AA) and protein pools by ^{3}H-leucine. Spermatocysts from Zones I, II, and III were exposed to the indicated concentrations of $HgCl_2$ or pCMBS (Zone 1 only) for 24 hours; label was present in the medium for the last hour of the experiment. Data given as mean per cent of paired controls (n=4), variability as SE bars.


MERCURIC CHLORIDE
ZONE 1
PROTEIN
AA POOL
% CONTROL VALUE
200
150
100
50
0
1
3
10
30
100
300
Hg CONCN (uM)


MERCURIC CHLORIDE
ZONE 2
% CONTROL VALUE
1
3
10
30
100
300
Hg CONCN (uM)


MERCURIC CHLORIDE
ZONE 3
% CONTROL VALUE
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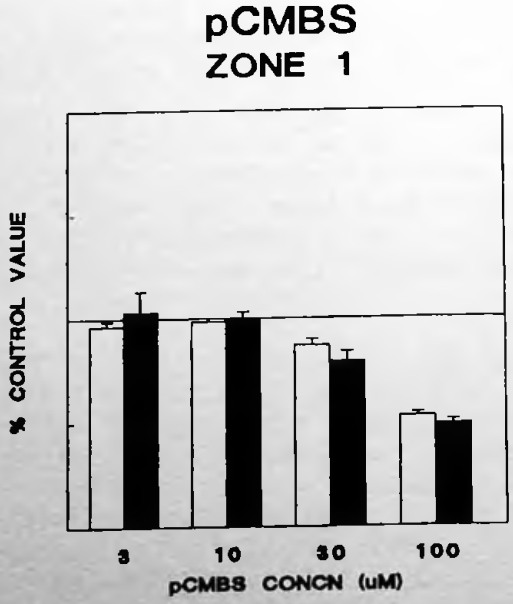
150
100
50
0
1
3
10
30
100
300
Hg CONCN (uM)


pCMBS
ZONE 1
% CONTROL VALUE
3
10
30
100
pCMBS CONCN (uM)