INDUCTION OF RAT LIVER ZINC METALLOTHIONEIN BY MERCURIC CHLORIDE [1]

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INTRODUCTION

Metallothionein is the name given to a class of low molecular weight, cysteine rich metalloproteins, which are found in various organisms after exposure to Cd(II) or Zn(II). Originally discovered as a cadmium rich protein from equine kidney (1), metallothionein has been purified to homogeneity from a variety of sources: human liver (2) and kidney (3), equine liver and kidney (4), rabbit liver (5) and kidney (6), chicken (7), pig (8), rat (9, 10), and calf and sheep liver (11). Metallothionein has been shown to be inducible in rat liver by zinc, cadmium, mercury and silver, this induction being sensitive to actinomycin D and cycloheximide (10).

Recently, we investigated the time course of induction by zinc and by cadmium of metallothionein in rat liver (12). Our results indicated a dramatic difference between the lifetimes of zinc induced metallothionein and of cadmium induced metallothionein, which was in support of the argument that metallothionein functions in zinc homeostasis and not in the detoxification of cadmium. It thus seemed of interest to us to investigate the time course of induction of metallothionein by another toxic metal, mercury, the results of which study are reported herein.

MATERIALS AND METHODS

Actinomycin D, Sephadex G-75, Tris-HCl, and sucrose were obtained from Sigma Chemical Co. Male, Sprague-Dawley rats (200-300 g) were obtained from Sasco, Inc., Omaha, Nebraska, and were maintained on Purina rat chow and tap water *ad libitum*. Mercuric chloride, reagent grade quality, was used for induction.

Metallothionein was induced using 0.5 mg Hg(II) per kg body weight as $HgCl_2$ in 2.0 ml saline, injected intraperitoneally. At appropriate times after induction the animals were killed by decapitation with a guillotine, their livers were excised, and these

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were individually quick-frozen on dry ice and stored at −20° for future use. Metallothionein was quantitated from each liver as described previously (12), expressing the results as μg metal in metallothionein per g liver wet weight. Zinc was determined by flame atomic absorption spectroscopy using a Perkin-Elmer Model 303, and mercury by flameless atomic absorption spectroscopy.

Actinomycin D was freshly prepared and dissolved in ethanol before use. It was diluted in 2.0 ml saline and administered at a dose of 1 mg per kg body weight.

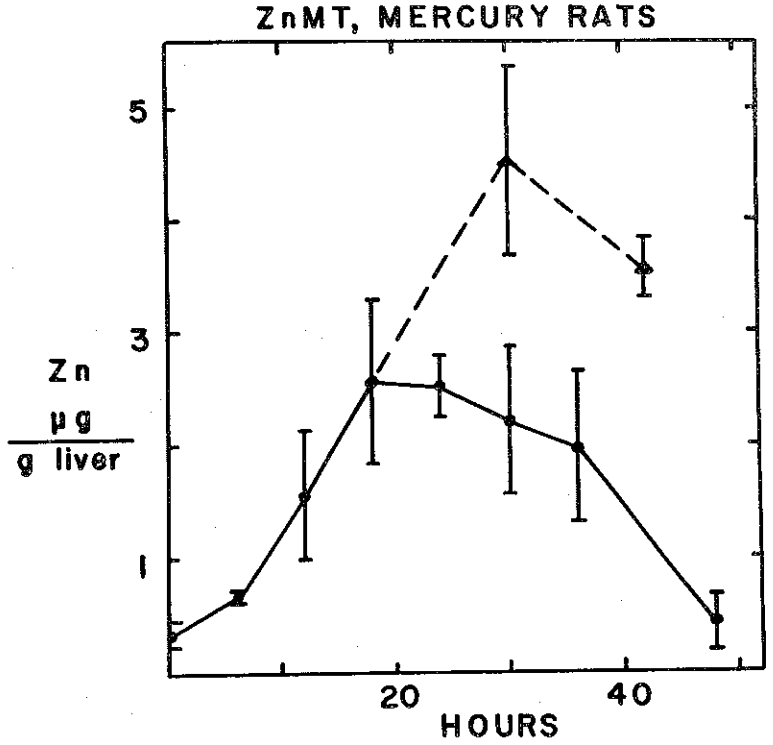

Figure 1. Rat liver zinc metallothionein after induction by mercury. Concentration of zinc metallothionein after a single dose of mercury (0.5 mg per kg body wt.) (●———●); at 18 hours after mercury administration a single dose of actinomycin D (1 mg per kg body wt.) was given and the concentration of zinc in metallothionein was determined (Δ - - - Δ). Numbers are expressed as the mean ± standard error for 3-4 animals.

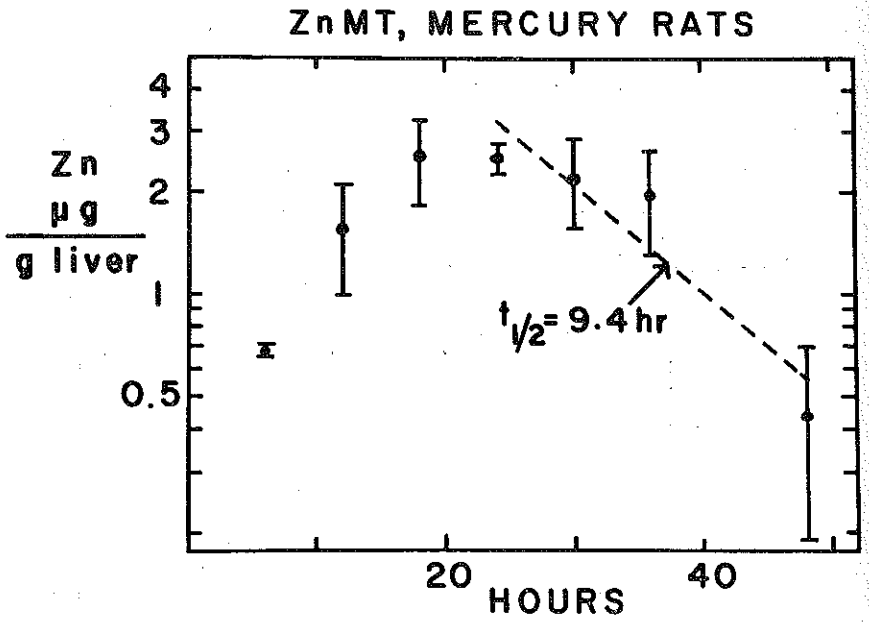

Figure 2. Semilog plot of the concentration of zinc in metallothionein after a single dose of mercury. Data were taken from the experiments in Figure 1. The line was calculated using a linear regression computor program.

RESULTS AND DISCUSSION

After treatment of rats with a single subtoxic dose of $HgCl_2$, zinc metallothionein was induced in their livers as shown in Figure 1 (●———●). The peak of induction occurred approximately 18 hours after administration of Hg(II), and the half time of decay was calculated to be 9.4 hours (Figure 2). No mercury was found to be associated with the zinc metallothionein, as purified on Sephadex G-75 and DEAE-cellulose. The behavior of the induction of zinc metallothionein in these animals following Hg(II) induction is reminiscent of that seen previously with zinc induction (12), as shown in Table I, and is distinctly different from that seen with the other toxic inducer, cadmium, which induces a metallothionein to which are bound both cadmium and zinc. Also, in our previous study of zinc and cadmium induction of metallothionein we noticed that zinc metallothionein, but not cadmium metallothionein, was capable of being "superinduced" by actinomycin D (12). "Superinduction" refers to the phenomena that occur when a high dose of actinomycin D is administered at the peak of induction of a protein, following a single dose of the inducer, in which an anomalously higher level of induction is attained post-actinomycin D treatment (13-15). The process is complicated and not well understood, but it is definitely not due to an increased level of messenger RNA (14), probably being related instead to some change in the rate of translation.

TABLE 1

Summary of Induction of Metallothionein (MT)

Treatment of Animals	ZnMT Peak	ZnMT $t_{1/2}$	CdMT Peak	CdMT $t_{1/2}$	Reference
Zinc(II)	18hr	10.1hr			(12)
Copper(II)	9hr	18.2hr			(12)
Cadmium(II)	24hr	4.53d	18hr	6.80d	(12)
Mercury(II)	18hr	9.4hr			(this study)

In this study the behavior of zinc metallothionein following Hg(II) treatment is typical of superinduction after administration of actinomycin D (1 mg per kg body wt.) at the peak of induction (18 hours) (Figure 1, Δ - - - Δ). As can be seen, levels of zinc metallothionein more than twice that of the control are attained 12 and 24 hours post-actinomycin D treatment.

The behavior of zinc metallothionein in rat liver following induction by a subtoxic dose of Hg(II) lends further credence to the idea that zinc homeostasis is central to the control of the level of this protein. Thus, exogenously administered Zn(II), Cu(II), Cd(II), and/or Hg(II) may result in an increased availability of intracellular zinc, which in turn may be inducing the production of metallothionein. The final metal content of the induced protein may only reflect the relative binding affinities for metallothionein of those metals present, cadmium being greater than zinc and copper and mercury not binding at all.

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