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LACTATION-INDUCED CADMIUM-BINDING PROTEINS

Maryka H. Bhattacharyya°, Daniel Solaiman+, Justine S. Garvey*, and Wesley Y. Miyazaki°

°Biological and Medical Research Division, Argonne National Laboratory, Argonne, IL 60439-4833
+Chemistry Department, Dusquesne University, Pittsburgh, PA 15282
*Biology Department, Syracuse University, Syracuse, NY 13244

One period of increased accumulation of toxic metals is during early childhood, when the gastrointestinal absorption and subsequent accumulation of toxic metals is significantly greater than in later life. A second period when uptake of toxic metals may be enhanced is during pregnancy and lactation, when demands for minerals by the mother are also increased.

Previously we have demonstrated striking increases during midlactation in ^{109}Cd absorption (2- to 3-fold) and retention by the duodenum (12-fold), kidney (5-fold), and mammary tissue (12-fold) of mouse dams receiving environmental levels of cadmium/^{109}Cd via drinking water, with little change in ^{109}Cd retention in liver and jejunum compared to nonpregnant controls[1-3]. Results are reported here of a study conducted to test the hypothesis that these increases in cadmium deposition during midlactation are caused by an induction of the metal-binding protein, metallothionein (MT). (The postulated induction of metallothionein would be a response to normal biochemical changes occurring during midlactation and not a response to cadmium administration, because, in the above studies of cadmium deposition during midlactation, the amounts of cadmium as ^{109}Cd received orally were negligible and would not have induced MT.)

A cadmium/hemoglobin (Cd/Hb) assay[4] for MT (which measures heat-stable cadmium binding capacity in tissues) was used to determine MT concentrations in the heat-treated supernatant fractions of kidney, liver, duodenum, and jejunum from five groups of B6CF1/ANL female mice (Table 1). Portions of the same supernatants were also analyzed for MT by radioimmunoassay (RIA) in the laboratory of Dr. Justine Garvey. As shown in Table 1, results obtained by RIA showed greater MT concentrations in liver and kidney from L13 and L20 mice and lower MT concentrations in all other tissues and groups than did the Cd/Hb assay. However, both assays demonstrated clear lactation-induced increases in MT concentrations in liver, kidney, and duodenum, with MT concentrations falling rapidly to control levels after weaning.

Table 1. Changes in Metallothionein Concentrations During Lactation in Mice[a]

Organ	Method	MT Concentration (ug/g tissues)				
		0-Time	NP	L13	L20	W5
Liver	Cd/Hb	11.5 ± 1.0	15.2 ± 1.4	64.2 ± 3.3	38.7 ± 2.6	11.2 ± 1.7
	RIA	2.7 ± 0.5	6.8 ± 1.2	161. ± 54.	32.5 ± 4.1	2.7 ± 0.9
Kidney	Cd/Hb	6.1 ± 0.6	11.3 ± 0.6	22.3 ± 1.2	21.7 ± 1.1	11.0 ± 0.6
	RIA	2.4 ± 0.1	5.9 ± 1.3	125. ± 25.	53.3 ± 8.9	3.7 ± 0.4
Duodenum	Cd/Hg	11.6 ± 1.5	19.1 ± 1.1	41.1 ± 3.8	37.7 ± 2.1	19.4 ± 1.9
	RIA	3.0 ± 0.4	2.1 ± 0.2	9.2 ± 2.6	5.2 ± 1.0	2.4 ± 0.2
Jejunum	Cd/Hb	6.5 ± 1.0	9.9 ± 0.5	13.9 ± 1.3	11.1 ± 0.8	10.7 ± 0.8
	RIA	<1.8	<1.7	5.4 ± 1.0	5.3 ± 0.7	<1.9

[a]Heat-treated supernatants were prepared from tissue homogenates from nonpregnant control mice on the day the experimental mice were mated, and 37 days later (0-Time and NP groups, respectively). Supernatants were also prepared from mouse dams on days 13 and 20 of lactation and day 5 after weaning (L13, L20, and W5 groups, respectively). Pups were weaned on lactation day 20. Values shown are means ± SE (n = 10).

Sephadex G-75 chromatography of the ^{109}Cd-containing supernatants from the Cd/Hb assays revealed that the majority of the ^{109}Cd radioactivity from all tissues of all groups appeared in a peak with a Ve/Vo of 2.1-2.2, the same elution position as we determined for Cd-induced mouse MT. The profiles from the L13 and L20 livers (but not the NP livers) contained additional smaller peaks: void volume, 5-10%; Ve/Vo = 1.6, 20-30% of the recovered ^{109}Cd (Fig. 1). Surprisingly, the 1.6 peak from liver showed a much higher specific binding to the MT antibody than did the 2.2 peak; the ratio of MT concentration estimated by RIA to that estimated by the Cd/Hb assay was ~ 1:1 for the 2.2 peak of liver and was ~ 10:1 for the 1.6 peak of liver. Possibly both the 1.6 and 2.2 peaks from liver are MT, with the 2.2 peak a smaller, more condensed form of the molecule.

In summary, we have demonstrated that metallothionein (or an MT-like cadmium-binding protein) increases in concentration in liver, kidney, and duodenum of mouse dams during lactation and decreases to levels in nonpregnant

controls by 5 days after weaning. We have evidence that the MT that appears in the liver of midlactating mice exists in two forms, a native form that chromatographs with a Ve/Vo = 1.6 that reacts strongly with MT antibody and a second form that appears with heating or storage in the freezer (data not shown), reacts less strongly with MT antibody, and chromatographs with a lower apparent molecular weight (Ve/Vo = 2.2). Additional investigations are needed to identify the role of this MT-like cadmium-binding molecule(s) that appears during a specific time course in the lactating mouse dam.

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REFERENCES

1. Bhattacharyya, M.H., B.D. Whelton, and D.P. Peterson, Toxicol. Appl. Pharmacol. 61, 335-342: 1981.s3
2. Bhattacharyya, M.H., B.D. Whelton, and D.P. Peterson, Toxicol. Appl. Pharmacol. 66, 368-375: 1982.
3. Bhattacharyya, M.H., D.A. Sellers, and D.P. Peterson, Environ. Res. 40, 145-154; 1986.
4. Eaton, D.L., and B.F. Tool, Toxicol. Appl. Pharmacol. 66, 134-142; 1982.

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