Metallothionein induction by cadmium and zinc in rat secretory organs

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Received 2 May 1988; accepted 10 June 1988

Summary. Metallothionein (MT) levels were determined in four secretory organs of the rat following administration of zinc (Zn) and cadmium (Cd). The concentrations of MT in the lacrimal, parotid and adrenal glands of untreated rats were in the range of 2.2–4.9 μg/g wet weight tissue while in the pancreas it was shown to be 15.2 μg/g. Injection of zinc at total doses of 16, 32 and 80 mg/kg resulted in a 1.8-, 3.2- and 5.9-fold increase in lacrimal MT content, respectively, while a 10.2- and 13.1-fold elevation was observed following treatment with 4 and 8 mg/kg of Cd, respectively. Similar findings were found in the adrenal gland. The parotid MT was elevated 5.9 and 17 times following Zn treatment at doses of 16 and 80 mg/kg respectively, whereas 4 mg/kg of Cd increased MT 14.4 times in this gland. Pancreatic MT was elevated by 39- and 40-fold after injection of Zn at doses of 16 and 32 mg/kg respectively, whereas 4 and 8 mg/kg of Cd caused a 9.8- and 17.9-fold induction, respectively. These results may indicate that secretory organs participate in metabolism of heavy metals in the mammalian body.
Key words. Metallothionein; heavy metals; secretory organs.

In the late 1950s Margoshes and Vallee demonstrated that cadmium was associated with a low molecular weight protein in the renal cortex of the horse[1]. This protein was termed metallothionein (MT) because of its high content of metal and cysteine residues[2,3]. MT was shown to be a cytoplasmic protein with molecular weight of about 6000[4]. This unique protein was induced by many chemicals, mainly heavy metals. The most powerful inducers of hepatic MT were shown to be cadmium and zinc[5,6], but other metals such as mercury and nickel were also effective in elevating hepatic MT content[5–8].

MT is thought to be a ubiquitous protein[4], and has been detected in various organs other than liver and kidney. Several groups have demonstrated enhanced MT synthesis as a response to heavy metal exposure in spleen, testes, intestine, brain, heart, and lung[5,9–11]. A markedly strong effect of zinc was demonstrated in the rat pancreas[5,10]. The metal-binding protein level in this secretory tissue was elevated by 40–80-fold after zinc administration at various doses. These results may indicate that MT and zinc are involved in the secretory process in the rat pancreas. However, in the mammalian body there are several secretory glands whose potential for MT induction is not known. Therefore, this study was designed to investigate the effect of zinc and cadmium on MT content in secretory tissues of exocrine function such as lacrimal and parotid glands, and of endocrine function such as adrenal gland.

Materials and methods. $ZnCl_2$ was purchased from Merck (Darmstadt, FRG) and $CdCl_2$ from Mallinckrodt (St. Louis, MO, USA). Hemoglobin and rabbit MT type II were obtained from Sigma (St. Louis, MO, USA). Radioisotopic cadmium (^{109}Cd) was purchased from New England Nuclear (Boston, MA, USA). Male Wistar-Sabra rats of about 350 g were housed at 22–24 °C, and a 12-h light-dark cycle was maintained. Animals were allowed free access to water and a commercial laboratory chow. Rats were injected i.p. with Zn (as $ZnCl_2$) at doses of 4, 8 and 20 mg/kg/day or Cd (as $CdCl_2$) at doses of 1 and 2 mg/kg/day for 4 days. Every dose group contained five rats. The metal salts were dissolved in saline (NaCl 0.9 %) and administered at volumes of 5 ml/kg for Zn, and 1 ml/kg for Cd. Controls were injected with equal volume of the vehicle. Two days following the last treatment the animals were sacrificed by heart puncture and the pancreas, parotid, lacrimal and adrenal glands were removed for MT determination.

Analytical methods. MT levels were determined by the hemoglobin radioassay method of Onosaka et al.[12] as described by Eaton and Toal[13]. Tissues were homogenized (1:3 w/v) in 10 mM Tris-HCl pH 7.4 at 4 °C for 30 s by a Kinematica homogenizer. The homogenates were centrifuged at 10 000 × g for 15 min at 4 °C. The obtained supernatants were heated (100 °C) for 2 min followed by 10 000 × g centrifugation at 4 °C. The supernatants (100 μl, appropriately diluted with Tris-HCl buffer) were incubated with 200 μl $CdCl_2$ 4 μM containing ^{109}Cd 1 μCi/ml and 100 μl 10 mM Tris-HCl buffer pH 7.4 in an Eppendorf polyethylene microcentrifuge tube. After 10 min of incubation at room temperature, 10 μl of NaOH 0.1 N were added following 100 μl of 2% bovine hemoglobin in water. The samples were incubated at 100 °C for 2 min, cooled on ice, and centrifuged at 10 000 × g for 2 min. Additional aliquot of 100 μl hemoglobin was added, heat denaturation and centrifugation were repeated. An aliquot of the supernatant fraction (300 μl) was counted for ^{109}Cd radioactivity. Appropriate blank (no sample) and total (no sample and hemoglobin) were included in each assay. MT concentrations were calculated by using purified rabbit MT type II as a standard in each experiment.

Results. The physiological levels of MT in four secretory organs of the rat were determined. In the lacrimal, parotid

Table 1. Zn- and Cd-induced MT in rat lacrimal and parotid glands

Metal	Total dose (mg/kg)	Lacrimal MT (μg/g)	Induction[a]	Parotid MT (μg/g)	Induction[a]
Saline	0	3.2 ± 0.4		2.2 ± 0.9	
Zn	16	5.7 ± 2.5[b]	1.8	13.0 ± 7.9[b]	5.9
Zn	32	10.1 ± 6.4[b]	3.2	ND	
Zn	80	18.9 ± 8.3[b]	5.9	37.5 ± 6.2[b]	17.0
Cd	4	32.6 ± 3.4[b]	10.2	31.7 ± 7.7[b]	14.4
Cd	8	42.0 ± 11.1[b]	13.1	ND	

Male rats were injected i.p. with Zn (as $ZnCl_2$) at 4, 8 and 20 mg/kg/day, or Cd (as $CdCl_2$) at 1 and 2 mg/kg/day for four days. Every dose group contained five animals. The rats were sacrificed 48 h following the last treatment and the lacrimal and parotid glands were removed for MT determination as described in Materials and Methods. Results are expressed as mean ± SD using the Mann-Whitney (2-tailed) U-test for statistical evaluation. ND: not determined; [a] calculated as the ratio between the treated and control animals; [b] $0.005 < p < 0.01$.

Table 2. Zn- and Cd-induced MT in rat adrenal and pancreas

Metal	Total dose (mg/kg)	Adrenal MT (μg/g)	Induction[a]	Pancreas MT (μg/g)	Induction[a]
Saline	0	4.9 ± 2.3		15.2 ± 8.6	
Zn	16	7.5 ± 3.2	1.5	588 ± 30[b]	39
Zn	32	18.0 ± 9.1[c]	3.7	597 ± 36[b]	40
Zn	80	ND		ND	
Cd	4	30.3 ± 8.3[b]	6.2	149 ± 48[b]	9.8
Cd	8	56.9 ± 14.2[b]	11.6	272 ± 218[b]	17.9

Male rats were injected i.p. with Zn (as $ZnCl_2$) at 4, 8 and 20 mg/kg/day, or Cd (as $CdCl_2$) at 1 and 2 mg/kg/day for four days. Every dose group contained five animals. The rats were sacrificed 48 h following the last treatment and the pancreas and adrenal glands were removed for MT determination as described in Materials and Methods. Results are expressed as mean ± SD using the Mann-Whitney (2-tailed) U-test for statistical evaluation. ND: not determined; [a] calculated as the ratio between the treated and control animals; [b] $0.005 < p < 0.01$; [c] $0.025 < p < 0.05$.

and adrenal glands MT concentrations were found to be 2.2–4.9 μg/g wet weight tissue while the pancreatic content of the metalloprotein was 15.2 μg/g (tables 1 and 2).

Treatment of rats with zinc and cadmium chloride resulted in significant elevations of MT concentrations in these secretory tissues. The metalloprotein content in the lacrimal gland was increased by 1.8-, 3.2- and 5.9-fold following injection of Zn at total doses of 16, 32 and 80 mg/kg, respectively (table 1). Cadmium was more potent than zinc in MT induction i.e. increase of 10.2 and 13.1 times was observed after treatment with Cd at total doses of only 4 and 8 mg/kg, respectively (table 1). Similar results were obtained in the rat adrenal gland (table 2). In the rat parotid, however, the effect of the metals was more pronounced. The cadmium-binding protein was elevated by 5.9- and 17-fold following treatment with 16 and 80 mg/kg of zinc, respectively, whereas increase of 14.4 times was observed following administration of Cd at as low a dose as 4 mg/kg (table 1). In the rat pancreas, however, Zn was a more effective MT inducer than Cd. There was 39- and 40-fold increase in pancreatic MT following treatment with Zn at doses of 16 and 32 mg/kg respectively, whereas induction by Cd in this organ was similar to that observed in other secretory tissues, i.e. elevation of 9.8- and 17.9-fold in rats exposed to 4 and 8 mg/kg, respectively (table 2).

Discussion. In the present study we have measured a) the physiological MT levels in the rat parotid, lacrimal, pancreas and adrenal glands, and b) the capacity of these secretory organs to synthesize MT following exposure to environmental heavy metals. The extent of MT induction caused by Cd was similar in the tested tissues. However, Zn-induced MT synthesis demonstrated significant organ differences. The scale of intensity of MT induction by Zn was as follows: pancreas > parotid > adrenal = lacrimal. Comparison between the ability of Zn and Cd to induce MT synthesis revealed that Cd was more potent than Zn in the lacrimal, adrenal and parotid, i.e. Cd was as effective as Zn but at significantly lower doses. This phenomenon was demonstrated in many tissues in which Cd was more potent than other metals including Zn in its inductive activity[14]. In contrast, the pancreatic MT was more affected by Zn (40-fold induction) than by Cd (18-fold). The organ and metal discrepancies can be a result of variations either in the capacity of different types of cells to respond to metals or in the bioavailability of the inducers to various secretory organs. Although MT is a ubiquitous metalloprotein throughout the mammalian body[1,4], its induction by metals was demonstrated in a few organs mainly in liver, kidney, intestine, lung, and pancreas[9,11]. These tissues contain secretory cells either with endocrine and exocrine functions such as the pancreatic cells or with other secretageous roles as the mucous cells in the gastrointestinal tract and respiratory system. In addition, hepatocytes and renal tissue are known to possess important secretory functions. It seems likely that induction of MT synthesis by metals is a common characteristic of secretory tissues. Since heavy metals are known to be accumulated in pancreas and salivary glands[15,16], it is assumed that organs with secretory functions are involved in metabolism of Zn as well as other metals. The increased tissue levels of metal-binding protein is an important physiological and toxicological factor by which the intracellular concentrations of free metals can be reduced and detoxified.

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0014-4754/88/090754-02$1.50 + 0.20/0