Minimal basal activity and lack of metal-induced activation of the metallothionein gene correlates with lobe-specific sensitivity to the carcinogenic effects of cadmium in the rat prostate.

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Metallothionein (MT), a high-affinity metal-binding protein, is known to detoxicate **cadmium** and may play an important role in **cadmium** carcinogenesis. In the rat, the ventral lobe of the **prostate** is sensitive to **cadmium** carcinogenesis, while the dorsolateral lobe is refractory. The possibility exists that the basis of this lobe-specific sensitivity may lie in the expression of the MT gene. Thus, the expression of the MT gene in lobes of the rat **prostate** was studied and, for comparative purposes, the expression of the MT gene in the liver, a tissue with well-defined high activity, was also assessed. MT gene expression was determined using a cDNA probe specific for MT-I, oligonucleotide probes specific for MT-I and MT-II, and an assay for **cadmium**-binding protein capacity. Basal levels of MT-I mRNA and **cadmium**-binding protein were much less in the ventral **prostate** than in the liver or dorsolateral **prostate**. **Cadmium**, given at a dose known to induce tumors of the ventral **prostate** (2.5 mumol/kg, sc), did not result in an increase in MT gene expression in the ventral **prostate**, as assessed by **cadmium**-binding protein levels or MT-I mRNA, over 72 hr. Small elevations of **cadmium**-binding protein capacity were detected at high doses of **cadmium** (25 and 40 mumol/kg) in the ventral **prostate** but no corresponding increases in MT mRNA were seen. In sharp contrast, hepatic MT gene expression was highly activated throughout the dosage range. Dose-response analysis 24 hr after **cadmium** administration (0.25 to 40 mumol/kg, sc) showed that MT-I and MT-II mRNA levels were increased in liver in a dose-dependent manner, while no evidence was found for MT gene activation in ventral **prostate**. In the dorsolateral **prostate** the high basal activity of the MT gene was shown, as assessed by MT-I and MT-II mRNA levels, which was not further elevated by **cadmium** treatments. **Cadmium** accumulation was much lower in the ventral **prostate** than in the liver. However, levels of **cadmium** that were sufficient to activate the hepatic MT gene had, in fact, reached the ventral **prostate**. Thus, the poor basal expression and lack of activation of the MT gene within the ventral lobe of the rat **prostate** may be the genetic basis to this tissue's sensitivity to the carcinogenic effects of **cadmium**.

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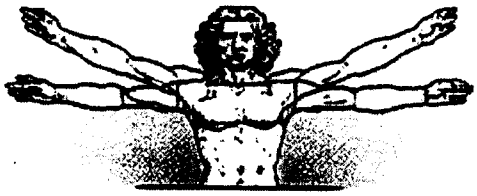
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Minimal Basal Activity and Lack of Metal-Induced Activation of the Metallothionein Gene Correlates with Lobe-Specific Sensitivity to the Carcinogenic Effects of Cadmium in the Rat Prostate

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Metallothionein (MT), a high-affinity metal-binding protein, is known to detoxicate cadmium and may play an important role in cadmium carcinogenesis. In the rat, the ventral lobe of the prostate is sensitive to cadmium carcinogenesis, while the dorsolateral lobe is refractory. The possibility exists that the basis of this lobe-specific sensitivity may lie in the expression of the MT gene. Thus, the expression of the MT gene in lobes of the rat prostate was studied and, for comparative purposes, the expression of the MT gene in the liver, a tissue with well-defined high activity, was also assessed. MT gene expression was determined using a cDNA probe specific for MT-I, oligonucleotide probes specific for MT-I and MT-II, and an assay for cadmium-binding protein capacity. Basal levels of MT-I mRNA and cadmium-binding protein were much less in the ventral prostate than in the liver or dorsolateral prostate. Cadmium, given at a dose known to induce tumors of the ventral prostate (2.5 μmol/kg, sc), did not result in an increase in MT gene expression in the ventral prostate, as assessed by cadmium-binding protein levels or MT-I mRNA, over 72 hr. Small elevations of cadmium-binding protein capacity were detected at high doses of cadmium (25 and 40 μmol/kg) in the ventral prostate but no corresponding increases in MT mRNA were seen. In sharp contrast, hepatic MT gene expression was highly activated throughout the dosage range. Dose–response analysis 24 hr after cadmium administration (0.25 to 40 μmol/kg, sc) showed that MT-I and MT-II mRNA levels were increased in liver in a dose-dependent manner, while no evidence was found for MT gene activation in ventral prostate. In the dorsolateral prostate the high basal activity of the MT gene was shown, as assessed by MT-I and M[illegible] mRNA levels, which was not further elevated by cadmium t[illegible]ments. Cadmium accumulation was much lower in the ve[illegible] prostate than in the liver. However, levels of cadmium that [illegible] sufficient to activate the hepatic MT gene had, in fact, rea[illegible] the ventral prostate. Thus, the poor basal expression and la[illegible] activation of the MT gene within the ventral lobe of the rat pros[illegible]tate may be the genetic basis to this tissue's sensitivity to the carcinogenic effects of cadmium. © 1995 Academic Press, Inc.

Cadmium is an important transition metal toxicant that continues to be a major environmental and occupational concern. Several comprehensive reviews exist on different aspects of cadmium toxicology, including carcinogenicity (Friberg *et al.*, 1986; IARC, 1976, 1993; Waalkes *et al.*, 1992; Goering *et al.*, 1995). Cadmium is a potent and effective carcinogen in rodents, and has recently been accepted by the International Agency for Research on Cancer as a category 1 (human) carcinogen (IARC, 1993).

Prostate cancer in humans is a common and lethal malignant disease. Of great concern is the fact that the incidence *and* the mortality rate of prostatic cancer continues to rise in many countries, including the United States (Stephens, 1991; Bosland, 1994). Epidemiologic studies have, on occasion, associated cadmium exposure with human prostatic cancer (Potts, 1965; Kipling and Waterhouse, 1967; Sorahan and Waterhouse, 1985; Lemen *et al.*, 1976; Kjellstrom *et al.*, 1979; Bako *et al.*, 1982; Abd Elghany *et al.*, 1990; West *et al.*, 1991). Other studies have found elevated levels of cadmium in the prostates of men with prostatic malignancies (Habib *et al.*, 1976; Feustel *et al.*, 1982, 1987; Feustel and Wennrich, 1984a,b; Ogunlewe and Osegbe, 1989).

In recent years there has been a remarkable increase in the experimental data supporting a potential role of cadmium in the etiology of prostate cancer. Chronic carcinogenicity studies in rats have established that the rat prostate is a target site for cadmium induction of neoplasia, whether

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exposure occurs orally (Waalkes and Rehm, 1992), parenterally (Waalkes *et al.*, 1988, 1989), or via direct injection into the prostate (Hoffman *et al.*, 1985a,b, 1988). Cadmium treatment can induce a full spectrum of prostatic proliferative lesions, including invasive, potentially metastatic, adenocarcinomas (Hoffman *et al.*, 1985a,b, 1988; Waalkes *et al.*, 1988, 1989). The rat prostate is a lobular structure with two major lobes, the dorsolateral and the ventral. The prostatic carcinogenic effects of cadmium in rats are uniformly confined to the ventral lobe and have not been detected in the dorsolateral lobe (Hoffman *et al.*, 1985a,b, 1988; Waalkes *et al.*, 1988, 1989; Waalkes and Rehm, 1992). Cadmium can also enhance the formation of chemically induced prostatic tumors in rats because the combination of im cadmium and 3,2'-dimethyl-4-aminobiphenyl given to rats acts synergistically to induce carcinomas of the prostate (Shirai *et al.*, 1993). Similarly, these tumors only developed in the ventral lobe (Shirai *et al.*, 1993), reinforcing evidence of lobe specificity of the carcinogenic or cocarcinogenic effects of cadmium. *In vitro* studies have demonstrated induction of malignant transformation in primary cultures of rat prostatic epithelial cells upon exposure to cadmium, but again only in cells isolated from the ventral lobe and not from the dorsolateral lobe (Terracio and Nachtigal, 1986, 1988). These findings are consistent with chronic rodent studies that unvaryingly show the ventral prostate is the target site for cadmium induction of prostatic tumors (Hoffman *et al.*, 1985a,b, 1988; Waalkes *et al.*, 1988, 1989; Waalkes and Rehm, 1992; Shirai *et al.*, 1993).

Metallothionein (MT) is a high-affinity metal-binding protein which is highly inducible by several metals, most notably cadmium and zinc (for review see Kagi, 1991; Waalkes and Goering, 1990). MT is thought to be an integral part of the primary cellular defense system against cadmium, and possibly other metals, and detoxication of cadmium is believed to occur through high-affinity sequestration (Waalkes and Goering, 1990; Templeton and Cherian, 1991). It is thought that MT levels play a critical role in determining the sensitivity or resistance of a given tissue or cell to various aspects of cadmium toxicity including aspects that may have bearing on carcinogenicity (Waalkes *et al.*, 1992).

Since the sensitivity to cadmium carcinogenesis within the rat prostate is clearly lobe specific (Hoffman *et al.*, 1985a,b, 1988; Waalkes *et al.*, 1988, 1989; Waalkes and Rehm, 1992; Shirai *et al.*, 1993), the possibility exists that the basis of this sensitivity may lie in the expression of MT. Thus we designed this series of experiments to define the expression of the MT gene in the major lobes of the rat prostate. For comparative purposes the expression of the MT gene in the liver, a tissue with well-defined high activity, was also assessed.

MATERIALS AND METHODS

Chemicals. Anhydrous cadmium chloride ($CdCl_2$) was purchased from Fisher Scientific (Fair Lawn, NJ). All other chemicals were obtained from Sigma Scientific (St. Louis, MO) except as indicated.

Animals, treatments, and tissue isolation. Adult male Wistar (WF/NCr) rats were obtained through FCRDC–Animal Production Area (Frederick, MD). Rats weighed between 250 and 400 g and were approximately 2.5–6 months old. Cadmium solutions were prepared fresh daily using $CdCl_2$ dissolved in physiologic saline. Cadmium was given as a single sc dose of 0.25, 0.5, 1.0, 2.5, 10, 20, or 40 μmol/kg in the dorsal thoracic midline at 4 ml/kg. The dose of 40 μmol Cd/kg, sc, is approximately a maximum tolerated dose of cadmium in rats based on weight gain and survival in chronic studies (Waalkes *et al.*, 1988). Rats were euthanized by CO_2 asphyxiation at various times after cadmium exposure and tissue samples were removed and quick-frozen in liquid nitrogen. A single liver (~250 mg) sample was obtained from each animal and the entire ventral or dorsolateral lobes of the prostate were removed and used as a single sample. Since cytosol or RNA isolation from the lobes of the prostate required the entire sample, each animals was used for either cytosol isolation or RNA isolation.

MT gene expression. RNA was extracted from tissues using RNAzol (Tel-Test, Inc., Friendswood, TX). RNA samples from at least two rats per group were isolated and duplicate aliquots of each sample were assayed. RNA samples were denatured using the glyoxal method (Thomas, 1980) and either resolved on a 1% agarose gel and transferred to Nytran (Schleicher and Schuell, Keene, NH) or slot-blotted onto Nytran. RNA integrity was determined by electrophoresis. Slot blots and Northern transfers were probed for MT-I gene expression using a ^{32}P-labeled cDNA probe specific for the rat MT-I gene. The plasmid was generously supplied by Dr. H. Herschman (Andersen *et al.*, 1983) and was labeled by nick translation using the service of Loftstrand Labs Limited (Gaithersburg, MD). In experiments where MT gene expression was determined using oligonucleotide probes, RNAzol-extracted samples were processed as described by Shiraishi and Waalkes (1994). RNA samples were denatured using the formaldehyde method (Davis *et al.*, 1986) and either resolved on a 1% agarose gel and subsequently transferred to Zeta-Probe membranes (Bio-Rad, Richmond, CA) or slot-blotted directly onto Zeta-Probe membranes. Membranes were probed for MT-I or MT-II gene expression using ^{32}P-labeled oligonucleotide probes specific for rat MT-I (5'-GAGGGCAGCAGCACTGTTCG-3'; Andersen *et al.*, 1986) or MT-II (5'-ACACCATTGTGAGGACGCCC-3'; McKim *et al.*, 1992). Probes were 5'-end-labeled using a T_4 polynucleotide kinase reaction (Loftstrand Labs Limited). The integrity of the RNA samples was confirmed by electrophoresis/Northern analysis. Slot blots were quantified by densitometry and RNA loading was standardized to β-actin gene expression (Shiraishi and Waalkes, 1994). Relative MT mRNA expression was defined as the ratio of MT mRNA content to β-actin content.

MT/cadmium-binding protein assessment. MT was estimated as heat-stable cadmium-binding protein capacity after using the cadmium–hemoglobin method of Onosaka *et al.* (1978) as modified by Eaton and Total (1982). Tissues were homogenized in 10 mM Tris–HCl, pH 7.4. Liver samples were homogenized at 1:4 (w/v), and prostate samples were homogenized in 1 ml. Postmitochondrial fractions were prepared by centrifugation (18,000*g* for 20 min). This assay will detect proteins other than MT (Waalkes and Perantoni, 1986) but is usually taken to reflect MT content in tissues where high levels are potentially found, such as liver. However, the precise nature of the protein is not defined by this assay and therefore results herein are expressed as cadmium-binding protein capacity.

Cadmium and zinc analysis. Tissue metal concentrations were measured by atomic absorption spectrophotometry using a Perkin–Elmer Model 5000 spectrophotometer after tissues were digested with concen-

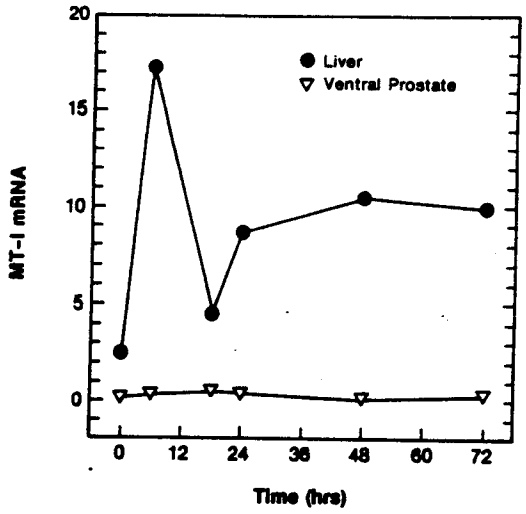


FIG. 1. Time course of MT-I mRNA accumulation in the liver and ventral prostate following exposure to cadmium. Rats were exposed at time zero to 2.5 μmol Cd/kg, sc, a dose known to induce ventral prostatic tumors. Relative MT-I mRNA expression was defined as the ratio MT-I mRNA content in relationship to β-actin mRNA content and presented as a ratio of densitometric signals. MT-I mRNA was determined by a ^{32}P-labeled cDNA probe specific for rat MT-I mRNA. All values were obtained by slot-blot analysis and represent the mean value of two animals.

trated nitric acid. Tissue cadmium levels were measured using a graphite furnace and zinc levels were measured by flame mode.

Statistical analysis. Unless otherwise stated, the data represent the mean ± SD of four separate determinations. Differences between treatment groups were evaluated statistically using analysis of variance (ANOVA) followed by Duncan's multiple range test for multiple comparisons or Student's *t* test for paired comparisons. Probability values of $p \leq 0.05$ were considered to indicate significant differences.

RESULTS

The time course of MT gene expression, as assessed by MT-I mRNA levels and cadmium-binding protein capacity in the liver and ventral prostate was investigated using a dosage of 2.5 μmol Cd/kg body wt. This dosage is below the level of cadmium known to induce acute testicular toxicity and dysfunction and will induce tumors of the ventral prostate in Wistar rats (Waalkes *et al.*, 1988, 1989).

The time course of MT gene expression after 2.5 μmol Cd/kg in the liver and ventral prostate was examined over the same 72-hr period using a cDNA probe specific for rat MT-I mRNA (Fig. 1). In the liver, a biphasic induction of the MT-I gene expression was detected. An approximately sevenfold increase above the basal MT-I mRNA level was observed at 6 hr following cadmium. At 18 hr, the MT-I mRNA levels decreased to approximately double the basal level but at 24 hr and thereafter levels again increased to approximately fourfold above the basal level. In marked contrast, no evidence for cadmium activation of MT-I gene expression was observed in the ventral prostate. Levels of MT-I mRNA in the ventral prostate remained constant at a very low level (Fig. 1).

The time course of MT protein induction was estimated as cadmium-binding protein capacity using the cadmium-hemoglobin method (Onosaka *et al.*, 1978). This assay can potentially detect proteins other than MT but is usually taken to reflect MT content in most tissues, such as liver, although the precise nature of the protein is not actually defined by this assay. In the liver, the single dose of cadmium resulted in an approximately 10-fold maximum increase in cadmium-binding protein compared to the level (Fig. 2). This increase was first measured at 6 hr persisted over the entire 72-hr period examined and reflects increases in MT gene translation product. In ventral prostate, this dose of cadmium did not result in detectable increase in cadmium-binding protein level addition, basal levels in the ventral prostate were far be those of the liver.

Cadmium accumulation in the liver and ventral pros following a single sc administration of 2.5 μmol Cd/kg body wt is presented in Table 1. The peak cadmium level in both tissues was observed at 6 hr. In general, these levels de creased slowly with time out to 48 hr after dosing. For a reference point, cadmium accumulation in the dorsolateral prostate is also presented in Table 1. Approximately three times as much cadmium is found in the dorsolateral pro tate as in the ventral prostate, the target for cadmium-in duced tumors. Although significant cadmium accumulation occurred in all tissues examined, the possibility exists

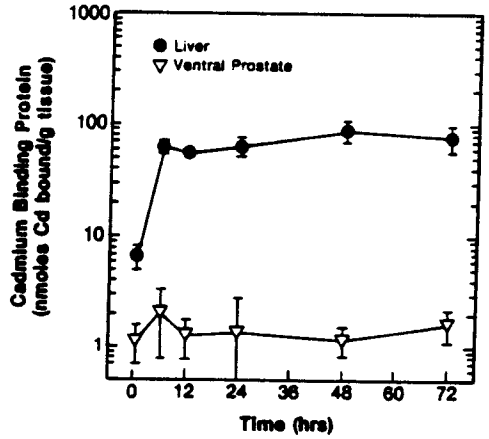


FIG. 2. Time course of cadmium-binding protein capacity in the liver and ventral prostate following exposure to cadmium. Rats were exposed at time zero to 2.5 μmol Cd/kg, sc, a dose known to induce ventral prostatic tumors. Cadmium-binding protein capacity was determined at various times after cadmium treatment by the method of Onosaka *et al.* (197 Data represent the mean ± SD (n = 4–8). Levels were significantly eleva ($p \leq 0.05$) over Time 0 in liver at all subsequent time points, while changes in capacity occurred in the ventral prostate.

TABLE 1
Accumulation of Cadmium in Various Tissues as a Function of Time

Time (hr)	Cadmium content (μg/g wet wt)[a]		
	Liver	Ventral prostate[b]	Dorsolateral prostate
Control	0.002 ± 0.003	0.001 ± 0.003	0.000 ± 0.00[c]
6	3.584 ± 0.563	0.070 ± 0.035	0.205 ± 0.084
12	3.143 ± 0.768	0.057 ± 0.017	0.152 ± 0.088
24	2.572 ± 0.403	0.044 ± 0.008	0.197 ± 0.084
48	2.892 ± 0.340	0.033 ± 0.009	0.110 ± 0.038

[a] Cadmium content determined following subcutaneous injection of cadmium chloride, 2.5 μmol/kg. Values expressed as the mean ± SD, $n = 4$.

[b] Levels in the ventral prostate were significantly lower ($p < 0.05$; Student's t test) than those in the dorsolateral prostate at all time points after cadmium exposure.

[c] Below limits of detection.

that insufficient cadmium reached the ventral prostate to induce MT expression.

Thus, the ability of cadmium exposure to induce MT gene expression in the ventral prostate was investigated over a wide dosage range of cadmium (1.0 to 40 μmol/kg). The sc dosage of 40 μmol Cd/kg is at or near the maximum tol-

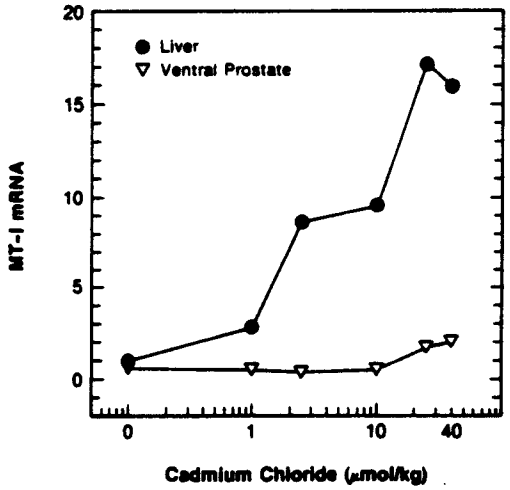


FIG. 3. Relative MT-I mRNA accumulation in the liver and ventral prostate following exposure to various doses of cadmium. Rats were exposed at time zero to 0, 1.0, 2.5, 10, 25, or 40 μmol Cd/kg, sc, and tissues were collected for RNA analysis 24 hr later. Relative MT-I mRNA expression was defined as the ratio of MT-I mRNA content in relationship to β-actin mRNA content and presented as a ratio of densitometric signals. MT-I mRNA was determined using a ^{32}P-labeled cDNA probe specific for rat MT-I mRNA. All values were obtained by slot-blot analysis and represent the mean value of two animals.

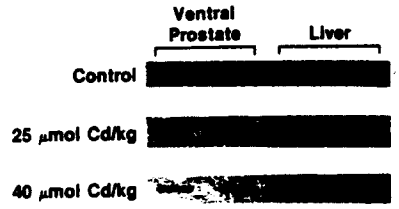


FIG. 4. Slot-blot analysis of MT-I mRNA accumulation in the liver and ventral prostate 24 hr following exposure to 25 or 40 μmol Cd/kg. Tissues for RNA were collected 24 hr after cadmium exposure. MT-I mRNA was determined by a ^{32}P-labeled cDNA probe specific for rat MT-I mRNA. Consistency in RNA loading was confirmed by β-actin mRNA (not shown).

erated dose in rats (Waalkes *et al.*, 1988). Dose-related activation of MT gene expression was investigated using both cDNA and oligonucleotide probes specific for rat MT mRNA. Analyses using a cDNA probe specific for rat MT-I are shown in Figs. 3 and 4. Exposure of rats to cadmium over a dosage range from 1.0 to 40 μmol/kg showed a clear dose-dependent activation of hepatic MT-I gene expression (Fig. 3) which was most pronounced at the highest doses (Fig. 4). Conversely, no evidence of activation of the MT-I gene was detected with any dose of cadmium in the ventral prostate (Fig. 3) even at levels at or near the maximum tolerated dose (Fig. 4).

Slot-blot analyses of mRNA from ventral prostate and liver were also conducted using oligonucleotide probes specific for rat MT-I and MT-II (Fig. 5). Clear dose-related increases in both hepatic MT-I and MT-II mRNA levels occurred with cadmium exposure compared to basal expression. Conversely, no evidence of increases in either MT-I or MT-II expression in the ventral prostate was seen, and the low basal mRNA levels remained unchanged with cadmium treatments. These data are consistent with those obtained using the cDNA probe for MT-I mRNA (see Figs. 1, 3, and 4).

MT-I and MT-II gene expression was also examined in the dorsolateral prostate (Fig. 5). Unlike the ventral prostate, basal levels of MT-I and MT-II gene expression in the dorsolateral lobe were very high, even greater than those observed in the liver. However, levels of MT mRNA in the dorsolateral prostate were not further increased by cadmium treatments. Consistent with the basal levels of MT gene expression, high levels of cadmium-binding protein were detected in the dorsolateral prostate (28.5 ± 4.31 nmol Cd bound/g tissue; mean ± SE, $n = 4$). In comparison, much lower basal cadmium-binding protein levels are detected in the liver (3.96 ± 0.51) and ventral prostate (1.21 ± 0.18). The high basal content of cadmium-binding protein in the dorsolateral prostate corresponded to a very high endogenous zinc content (139.3 ± 9.7 μg/g tissue; mean ± SE,

n = 11). Zinc tissue levels were much more modest in the liver (21.3 ± 0.84) and ventral prostate (13.7 ± 2.2).

The effects of exposure to cadmium on cadmium binding protein capacity, as assessed 24 hr after exposure, are presented in Fig. 6. Clear dose-dependent increases in MT occurred in the liver which peaked at 25 μmol Cd/kg, consistent with the data for MT mRNA accumulation (see Figs. 3, 4, and 5). In contrast, at dosages up to 10 μmol cadmium/kg the levels of cadmium-binding protein in the ventral prostate were not different from basal levels. At dosages of 25 and 40 μmol/kg slight increases in cadmium-binding protein capacity were detected. These increases do not, however, correspond with any modifications in MT mRNA levels (Figs. 4 and 5).

The accumulation of cadmium in liver and ventral prostate and dorsolateral prostate following subcutaneous administration of cadmium is presented in Table 2. A dosage-dependent increase in cadmium accumulation occurred in liver and ventral prostate at dosage up to 25 μmol Cd/kg, while similar levels were attained following administration of either 25 or 40 μmol Cd/kg. The similar cadmium levels obtained in the liver at 25 and 40 μmol/kg are consistent with the similar levels of MT gene expression (Figs. 3, 4, and 5) and protein synthesis (Fig. 6). A dosage-dependent increase in cadmium accumulation occurred in the dorsolateral prostate over the entire range tested. Accumulation in the liver was much greater than in the ventral prostate. Administration of cadmium had no effect on basal zinc levels in either tissue (data not shown).

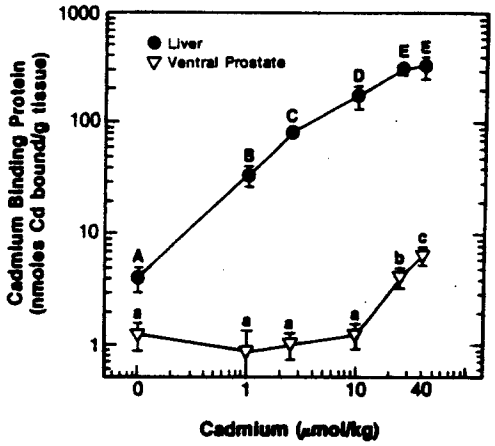


FIG. 6. Cadmium-binding protein capacity in the liver and ventral prostate following exposure to various doses of cadmium. Rats were exposed at time zero to 0, 1.0, 2.5, 10, 25, or 40 μmol Cd/kg, sc. Cadmium-binding protein capacity was determined 24 hr after cadmium treatment by the method of Onosaka *et al.* (1978). Data represent the mean ± SD (n = 4–8) and differing superscripts (A, B, C, D, E for liver; a, b, c for ventral prostate) indicate significant ($p \leq 0.05$) differences. Levels were significantly elevated ($p \leq 0.05$) over control in liver at doses of cadmium. No changes in capacity occurred in the ventral prostate until the cadmium dose exceeded 10 μmol/kg.

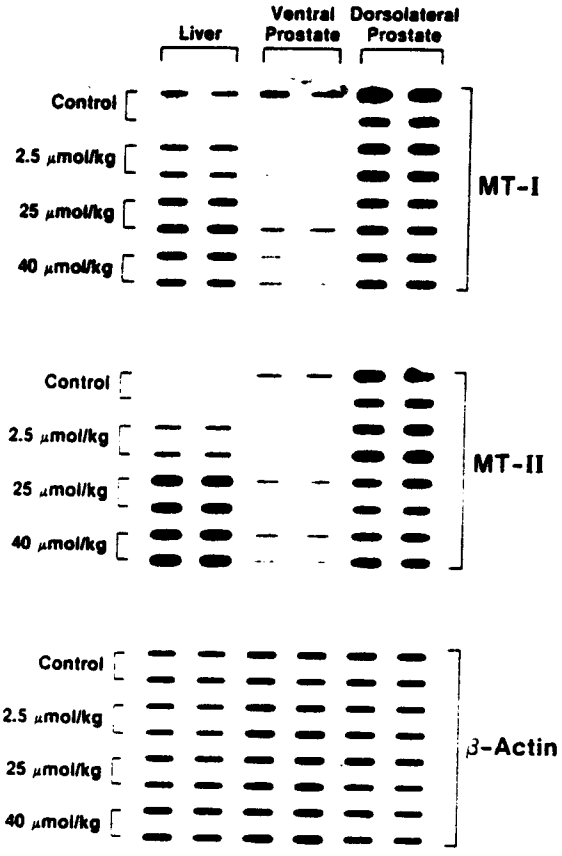


FIG. 5. MT-I and MT-II mRNA accumulation in the liver, ventral prostate, and dorsolateral prostate after exposure to various doses of cadmium. Rats were exposed at time zero to 0, 2.5, 25, or 40 μmol Cd/kg, sc, and tissues were collected for RNA analysis 24 hr later. MT-I and MT-II mRNA were determined using specific oligonucleotides (see Materials and Methods). Consistency in RNA loading was confirmed by β-actin mRNA.

TABLE 2
Accumulation of Cadmium in Various Tissues as a Function of Dose

Dose (μmol/kg)	Cadmium content (μg/g wet wt)[a,b]		
	Liver	Ventral prostate	Dorsolateral prostate
Control	0.002 ± 0.003	0.001 ± 0.003	0.000 ± 0.00[c]
2.5	2.572 ± 0.40	0.044 ± 0.008	0.197 ± 0.08
10	7.876 ± 1.086	0.251 ± 0.199	0.648 ± 0.09
25	15.955 ± 2.368	0.820 ± 0.370	1.303 ± 0.36
40	17.258 ± 5.33	0.899 ± 0.236	2.355 ± 0.32

[a] Cadmium content determined 24 hr following subcutaneous exposure.
[b] Values expressed as the mean ± SD, n = 4.
[c] Below limits of detection.

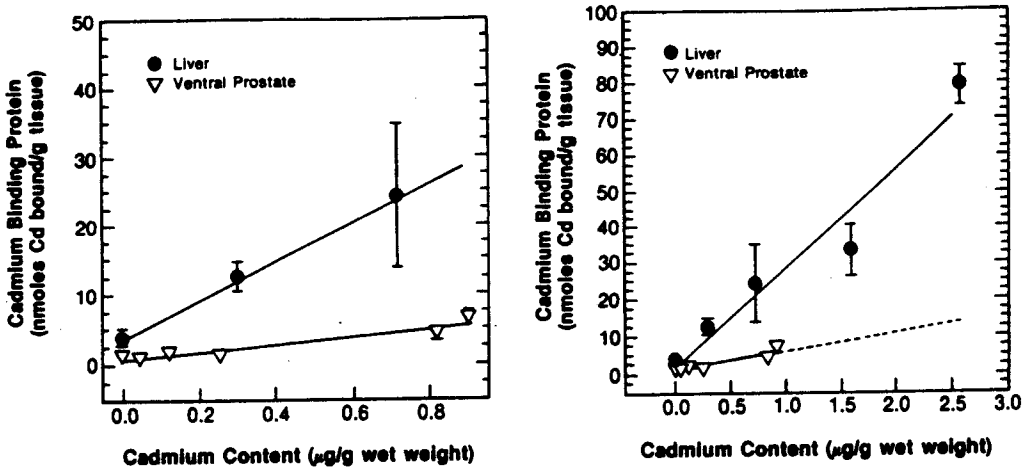


FIG. 7. Correlation of cadmium-binding protein capacity and cadmium tissue levels in liver and ventral prostate. The data obtained from the tissue analysis for cadmium-binding protein (Fig. 6) and for tissue cadmium content (Table 2) were correlated and are presented (right). In addition, supplementary data were generated to provide the liver with tissue cadmium burdens similar to that obtained in the ventral prostate (left; see Materials and Methods). Data represent the mean ± SD (n = 4–8).

Since the liver accumulated greater amounts of cadmium than the ventral prostate, studies were designed to obtain hepatic cadmium levels in the range of those occurring in the ventral prostate, by reduction of cadmium dosage, and the expression of the hepatic MT gene was then assessed. Rats were given 0.25, 0.5, and 1 μmol Cd/kg body wt, and liver MT levels and cadmium content were determined at 24 hr. These data were combined with the previous data and cadmium-binding protein levels were correlated with cadmium tissue content (Fig. 7). There were clear, tissue content-related increases in hepatic cadmium-binding protein levels when concentrations of cadmium were obtained in the range of those in the ventral prostate. However, this range of cadmium tissue levels fail to produce appreciable amounts of the cadmium-binding protein in the ventral prostate.

DISCUSSION

The present results indicate that the MT gene is only minimally active and is not inducible by cadmium within the ventral lobe of the rat prostate. In contrast, the MT gene in the liver is highly inducible by cadmium. More importantly, the MT gene in the dorsolateral prostate was shown to have a very high basal activity. In chronic rodent studies, cadmium-induced tumors of the rat prostate occur exclusively within the ventral lobe and have not been seen within the dorsolateral lobe (Waalkes *et al.*, 1988, 1989; Waalkes and Rehm, 1992; Hoffmann *et al.*, 1985a,b, 1988; Shirai *et al.*, 1993). Within the ventral lobe of the rat prostate cadmium can induce a full spectrum of prostatic proliferative lesions, including invasive, potentially metastatic, adenocarcinomas (Waalkes *et al.*, 1988, 1989; Waalkes and Rehm, 1992; Hoffmann *et al.*, 1985a,b, 1988; Shirai *et al.*, 1993). These tumors induced in rats by cadmium histologically resemble a certain subset of human prostate neoplasia (McNeal *et al.*, 1986; Wernert *et al.*, 1990; Waalkes and Rehm, 1994). Although cadmium can induce prostatic tumors in the rat when given by various routes (Waalkes *et al.*, 1988, 1989; Waalkes and Rehm, 1992; Hoffmann *et al.*, 1985a,b, 1988) or can function as a cocarcinogen when given together with organic carcinogens (Shirai *et al.*, 1993) the tumors produced are exclusively of the ventral lobe. Thus, the ventral-lobe-specific sensitivity to cadmium carcinogenicity clearly is correlated with a lobe-dependent lack of expression of the MT gene.

MT is thought to be a primary component of native or acquired cellular tolerance to toxic effects of cadmium (Templeton and Cherian, 1991; Vallee and Maret, 1993; Waalkes and Goering, 1990). Upon cadmium exposure the MT gene responds by synthesis of a high-affinity metal-binding protein (Durnam and Palmiter, 1981) and detoxication of cadmium is believed to occur subsequently through sequestration of the metal (Templeton and Cherian, 1991; Goering *et al.*, 1994). It appears that the activity of the MT gene plays a critical role in determining the sensitivity or resistance of a given tissue or cell to various aspects of cadmium toxicity including aspects of probable importance in carcinogenicity (Waalkes *et al.*, 1992). For instance, in R2C cells, a testicular Leydig cell line, the MT gene appears to be quiescent and does not respond to inducing stimuli, and these cells are highly sensitive to cadmium-induced DNA damage (Shiraishi *et al.*, 1995). Furthermore, in TRL 1215 cells, a liver-derived cell line with

an active and inducible MT gene, stimulation of MT expression prior to exposure to cadmium markedly reduces cadmium-induced genotoxicity (Coogan *et al.*, 1992, 1994). Cadmium exposure will also activate expression of the proto-oncogenes c-*jun* and c-*myc* in a rat myoblast cell line (Jin and Ringertz, 1990), and preactivation of MT gene expression by zinc exposure markedly reduces the stimulation of proto-oncogene expression by subsequent cadmium exposure (Abshire *et al.*, 1994).

Thus, the inactivity of the MT gene may well allow a greater rate of carcinogenic transformation of target cells and/or allow transformation to occur at relatively low concentrations of cadmium. It has been shown that *in vitro* cadmium exposure can induce malignant transformation in primary cultures of rat ventral prostatic epithelial cells (Terracio and Nachtigal, 1986, 1988). Although the ventral prostatic epithelial cells undergo transformation with exposure to cadmium, dorsolateral epithelial cells do not (Terracio and Nachtigal, 1986, 1988). Malignant transformation was confirmed in that cadmium-transformed ventral prostatic epithelial cells produce malignant tumors upon injection into newborn rats, while untreated cells are not affective (Terracio and Nachtigal, 1988). This suggests that cadmium carcinogenicity in the prostate is due to direct interactions with epithelial cells of the ventral prostate. Interestingly, these primary cultures of rat ventral prostatic epithelial cells were also highly sensitive to cadmium cytotoxicity (Terracio and Nachtigal, 1986, 1988), a common finding in cells where the MT gene is inactive or otherwise poorly expressed (Compere and Palmiter, 1981; Shirashi *et al.*, 1995). These findings are consistent with the present results indicating a lobe-specific deficiency in expression of the major cellular tolerance system to cadmium and with chronic rodent studies that uniformly show the ventral prostate as the target site for cadmium induction of prostatic tumors (Waalkes *et al.*, 1988, 1989; Waalkes and Rehm, 1992; Hoffmann *et al.*, 1985a,b, 1988; Shirai *et al.*, 1993).

Other recent evidence indicates that MT may be poorly expressed in the ventral lobe of the rat prostate, and the MT gene in this specific lobe is generally unresponsive to metal stimuli. Very low constitutive levels of MT mRNA are detected in the ventral prostate by *in situ* hybridization techniques (Tohyama *et al.*, 1993). Assessment of MT protein levels by Western blot analysis again indicates basal levels in the ventral prostate at or near detection limits (Timms and Hagen, 1989). In the present study increased MT gene expression in the ventral prostate does not occur with cadmium treatment, despite the fact that cadmium concentrations in this lobe can be in excess of those that cause marked stimulation of hepatic MT gene expression. In contrast, high basal MT mRNA levels are detected in the dorsolateral prostate, a lobe refractory to cadmium carcinogenicity, by standard molecular techniques (present study) and by *in situ* hybridization (Tohyama *et al.*, 1993). It is noteworthy that MT gene expression is clearly not stimulated in the rat ventral prostate by the subcutaneous doses of cadmium that are carcinogenic within the ventral prostate (1–2.5 μmol Cd/kg, sc; Waalkes *et al.*, 1988, 1989). It is also significant that exposure to cadmium doses that are carcinogenic to the ventral prostate resulted in the accumulation of approximately three times more cadmium in the dorsolateral lobe than in the ventral lobe. Clearly, the toxicokinetics of cadmium do not dictate the lobe-specific sensitivity in the prostate. In apparent conflict with the present study, Suzuki *et al.* (1993) found higher total doses of cadmium (16–48 μmol/kg, sc) apparently stimulated production of a protein recognized as MT by radioimmunoassay in the ventral prostate, but only when cadmium was given with concurrent testosterone treatments. Our findings indicate that a cadmium-binding protein did accumulate in the ventral prostate with doses of cadmium in excess of 20 μmol/kg but this accumulation was not related to activation of the MT gene, as assessed by the absence of an elevation of MT gene transcript. The discrepancy between the current results and the results of Suzuki *et al.* (1993) cannot be determined at present but there are indications that testosterone itself may be a primary stimulant of prostatic MT gene expression, at least in the dorsolateral lobe (Tohyama *et al.*, 1993).

Also in agreement with the molecular biological analysis of the current study, a number of studies have shown that, under normal circumstances, there is little immunohistochemical evidence that MT is present within the ventral lobe of the rat prostate, although the dorsal prostate often shows significant staining. Umeyama *et al.* (1987) reported the ventral lobe of the rat prostate was immunohistochemically negative for MT and staining was unchanged by cadmium exposure. Similarly, Nishimura *et al.* (1990) detected staining for MT in the ventral lobe of untreated rats only as a rare event and this pattern was unchanged by zinc treatments. Suzuki *et al.* (1993) found no immunohistochemical evidence of MT in the ventral prostate from untreated rats, and although staining occurred after cadmium treatment it was primarily in basal cells and not in epithelial cells. Timms and Hagen (1989) found little evidence of MT staining in the epithelium of ventral prostate in 14-day-old rats, although some staining occurred in stromal components, and zinc and cadmium treatments had no effect on these levels. Immunohistochemical semiquantitation of MT in the rat prostate can be highly variable and dependent on the technique utilized (Bataineh *et al.*, 1986) and although the results of immunohistochemical studies are variable, in general they indicate MT is poorly expressed in the ventral lobe of the rat prostate. Immunohistochemical

localization of MT in the human prostate indicates that the presence of MT protein varies widely based on location within the gland but that large portions of the tissue show very weak staining intensity (Suzuki *et al.*, 1991). The expression of MT mRNA in the human prostate has not been, as yet, defined.

A previous study in our laboratory, designed to determine the chemical nature of the cadmium-binding proteins in the rat prostate, failed to find MT in either of the ventral or dorsolateral lobes of the rat prostate (Waalkes and Perantoni, 1989). A low-molecular-weight cadmium-binding protein was isolated from the prostate by standard techniques for MT isolation, but when further chemically analyzed it proved quite distinct from MT in amino acid composition and contained much less cysteine and amino acids generally absent from MT (Waalkes and Perantoni, 1989). The absence of biochemically defined MT in the ventral prostate is consistent with the results from the present study, and with the investigations of others (Umeyama *et al.*, 1987; Nishimura *et al.*, 1990; Tohyama *et al.*, 1993). However, for the dorsolateral prostate the basis of the discrepancies between these previous data (Waalkes and Perantoni, 1989) and immunohistochemical and radioimmunoassay data, as well as the molecular biological results of the present study, is not immediately apparent. Our work demonstrates that a high level of a cadmium-binding protein, as determined by the cadmium–hemoglobin method of Onosaka *et al.* (1978), exists in the rat dorsolateral prostate. The presence of this protein correlates with the high basal level of MT gene expression in this lobe, thus suggesting that this protein is indeed MT. Northern analysis confirmed that each probe recognized a single mRNA band with the approximate size of MT (~550 bases) and probe recognition was determined after stringent washing, indicating strong homology to MT. It is quite unlikely that a protein as divergent from MT as the one initially characterized (Waalkes and Perantoni, 1989) would be a translational product of a gene strongly homologous to MT. Further investigation is required to clarify the nature of the cadmium binding protein(s) of the dorsolateral prostate.

In summary, in all studies thus far, cadmium induction of prostatic tumors occurs only within the ventral lobe of the rat prostate, while the dorsolateral lobe appears resistant. The poor basal expression and the lack of metal-induced stimulation of the MT gene specifically in the ventral lobe and the high basal expression in the dorsolateral lobe correlates with the lobe-specific susceptibility to the carcinogenic effects of cadmium in the rat prostate. The lack of responsiveness in this important cellular detoxication system for cadmium appears to be an important factor in determining final sensitivity to cadmium carcinogenesis, at least in the rat ventral prostate. The precise relevance of these findings to the human prostate is unknown, although the poor expression of MT, as assessed immunohistochemically, in most areas of the human prostate gland (Suzuki *et al.*, 1991) may be of importance considering the possible association between cadmium exposure and human prostate cancer.

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