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Expression and Regulation of Metallothioneins in the Rat Epididymis

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ABSTRACT: Metallothioneins (MTs) are cytosolic proteins involved in cellular stress responses. The objectives of this study were to determine which epididymal cells express MTs, how they are regulated, and whether mRNA levels for 3 MT isoforms (MT I, MT II, and MT III) are modulated by heavy metals. MT expression was noted mainly in basal cells of all epididymal regions but not in all basal cells of any given region. MT I mRNA levels were highest in the testis, followed by levels in the corpus, cauda epididymidis, liver (positive control), caput epididymidis, initial segment, seminal vesicles, and ventral prostate. MT II mRNA levels were also highest in testis, followed by levels in the cauda, corpus, liver, caput, and initial segment, but they were undetectable in the seminal vesicles and ventral prostate. MT III mRNA levels were highest in the caput followed by testis and initial segment. Orchidectomy and orchidectomy with testosterone replacement experiments showed that immunoreactive MT in all epididymal segments was androgen dependent. Epididymal MT I mRNA levels were dependent on androgens in all segments except the corpus. MT II mRNA levels were androgen dependent only in the initial segment and corpus. MT III mRNA levels in the initial segment were not altered by orchidectomy but increased significantly in testosterone-treated rats. In the caput, MT III mRNA levels decreased following orchidectomy, but control levels were maintained by testosterone. In cadmium-injected rats, MT I mRNA levels were significantly increased in the testis and initial segment, but there were no effects in the liver and other epididymal regions. MT II mRNA levels were increased by more than eightfold in the liver and by three- to fourfold in the initial segment and caput. In the corpus, MT II mRNA levels were decreased by cadmium treatment. MT III mRNA levels were unaltered by cadmium treatment. In conclusion, all 3 MT transcripts are present in high abundance in the epididymis. Furthermore, MT is expressed mainly in basal cells with regulation by testosterone. Heavy metal induction appears to affect the proximal regions of the epididymis.

Key words: Androgen, cadmium.

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Metallothioneins (MTs) are low–molecular weight cytoplasmic proteins rich in cysteine residues. These cysteine residues can bind heavy metals, especially zinc and cadmium, suggesting that they are important for metal homeostasis (Hammer, 1986). MTs are also involved in scavenging cellular free oxygen radicals (Karin, 1985; Lazo and Pitt, 1995; Hurnanen et al, 1997). There are 4 isoforms of MT (Oh et al, 1978; Hammer, 1986; Palmiter, 1998). Most of the research attention has focused on the MT I and MT II. These 2 isoforms of MT are expressed together in most tissues, and their cellular levels can be increased by a variety of agents, including heavy metals, glucocorticoids, lipopolysaccharides (LPS), and interferon. The sensitivity of these proteins to class II metals has led to their use as biomarkers of heavy metal exposure, particularly in the liver and kidney (De et al, 1991; Palmiter, 1998). MTs protect cells from heavy metals by sequestering the metals, thereby reducing their bioavailability and consequently, their cytotoxicity (Jacob et al, 1999).

In contrast to MT I and MT II, the expression of MT III and MT IV is highly tissue specific. MT III is expressed in the brain, predominantly in neurons, and in the choroid plexus and testis (Masters et al, 1994; Moffat and Seguin, 1998; Dufresne and Cyr, 1999). MT IV is expressed in differentiating stratified squamous epithelial cells (Quaife et al, 1994). It has been reported that MTs are present throughout the male reproductive tract, including the epididymis, prostate, and seminal vesicles (De et al, 1991; Moffatt and Seguin, 1998). MT I and MT II transcripts are present in both pachytene spermatocytes and round spermatids, whereas low MT levels were observed in Sertoli cells (De et al, 1991) and interstitial Leydig cells (McKenna et al, 1996; Moffatt and Seguin, 1998). MT III mRNA transcripts have been identified in the epididymis and prostate (Moffatt and Seguin, 1998), although there is no information on the regulation of MTs in the different epididymal regions. In the prostate, MT I

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and MT II are present in the dorsolateral lobes, and their expression is androgen dependent (Ghatak et al, 1996; Tohyama et al, 1996; Moussa et al, 1997; Lee et al, 1999). The low induction of MT levels by heavy metals, LPS, or both in the prostate and testis suggest that these tissues are not particularly sensitive to MT inducers as compared with the tissues of the liver or kidney (De et al, 1991; McKenna et al, 1996). Overexpression of MT I by the testis of transgenic mice did not protect the testis from cadmium-induced necrosis, suggesting other mechanisms of action of cadmium on the testis (Dalton et al, 1996).

There have been few studies to date that have examined the capacity of epididymal epithelial cells to detoxify environmental toxicants and protect maturing spermatozoa. This information is particularly important given that spermatozoa are stored in the cauda epididymidis and could represent targets for xenobiotics. Heavy metals such as cadmium and mercury are known male reproductive toxicants (Chowdhury et al, 1989; Ragan and Mast, 1990). Human and wildlife exposure to heavy metals are well established (Chan et al, 1995; Telisman et al, 2000; Zenes, 2000). Given the fact that heavy metals act on tissues of the male reproductive tract, it is important to understand the regulation of MTs in these tissues and whether or not these can be induced by exposure to heavy metals, thereby protecting the cells from these toxicants.

The objectives of this study were to immunolocalize MT; to determine MT I, MT II, and MT III mRNA levels in the epididymis relative to other male reproductive organs; to assess their regulation by androgens, and finally, to determine whether cellular levels of MT mRNA can be increased by a strong MT inducer, cadmium.

Materials and Methods

Animals

Adult male Sprague-Dawley rats (350–400 g) were purchased from Charles River Canada Inc (St Constant, Canada). Rats were maintained under a constant photoperiod of 12 hours light:12 hours dark and received food and water ad libitum. All the procedures used on animals in this study were approved by the University Animal Care Committee.

Experimental Protocols:

MT mRNA Levels Along the Male Reproductive Tract—Adult male rats were euthanized with CO_2. Testes, epididymides, seminal vesicles, and ventral prostate were removed by dissection. The epididymis was subdivided into 4 separate segments (initial segment, caput, corpus, and cauda epididymidis; Figure 1). Liver was used as a positive control. All tissues were frozen in liquid nitrogen and stored at −80°C. Tissues were later used for RNA isolation and Northern blot analyses.

Immunolocalization of MT—The immunocytochemical localization of MT was done using an affinity-purified monoclonal anti-MT antibody (10 μg/mL; Zymed Laboratories, Seattle, Wash). This antibody recognizes both MT I and MT II.

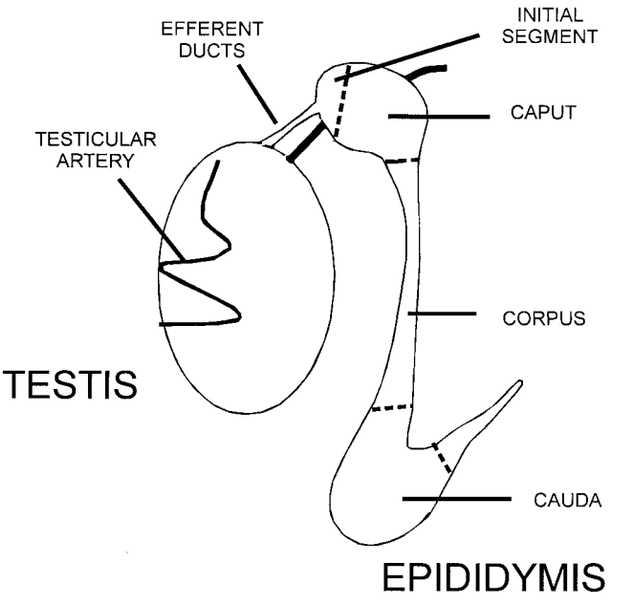

Figure 1. Schematic diagram of the testis and epididymis indicating the different regions (initial segment, caput, corpus, and cauda epididymidis) of the epididymis that were used in the present experiments.

To determine the immunocytochemical localization of MT, adult rats were anesthetized with sodium pentobarbital, and their reproductive tracts were fixed by retrograde perfusion through the abdominal aorta with Bouin fixative for 10 minutes. After fixation, the epididymis was removed, cut, and left in Bouin fixative for 24 hours before being dehydrated and embedded in paraffin. The epididymis was cut along its longitudinal axis so as to include each of its major regions, as depicted by Hermo et al (1994). Antibody binding to MT was detected by the horseradish peroxidase method outlined by Cyr et al (1992). Slides incubated with normal rabbit antisera were used as a negative control.

Regulation by Androgens—To assess whether epididymal MTs were regulated by androgens, adult rats were anesthetized with an intraperitoneal injection of ketamine/xylazine (50:10 mg/kg) and orchidectomized via a scrotal incision. Testosterone-filled polydimethylsiloxane capsules were prepared according to the method of Stratton et al (1973); these have well-characterized steroid release rates (Brawer et al, 1983). Orchidectomized rats were implanted with either an empty 2.5-cm capsule or 3 capsules of 6.2 cm each (total, 18.6 cm) of testosterone. The 18.6-cm capsule of testosterone mimics epididymal testosterone levels, which are 10 times greater than blood levels. Carrier rats were implanted with testosterone capsules for 3 days before the start of the experiment so that the initial surge of testosterone release would be complete and that the newly made capsules would have a constant testosterone release rate. The implants were removed from the carrier, cleaned, and inserted subcutaneously on the backs of the experimental animals at the time of orchidectomy. Rats were sampled at 3, 7, 14, and 21 days after surgery, and immunocytochemical procedures were performed

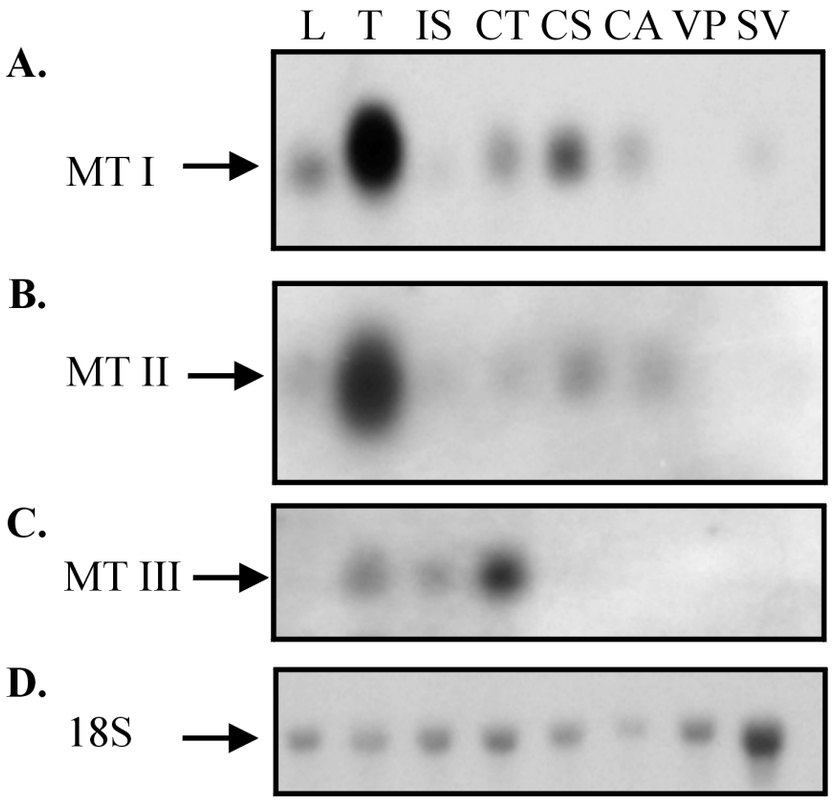

Figure 2. Northern blots of metallothioneins I (MT I), II (MT II), and III (MT III) mRNA along the male reproductive tract and liver. Total cellular RNA was isolated from the liver (L), testis (T), epididymal segments (initial segment [IS], caput [CT], corpus [CS], and cauda [CA] epididymidis), ventral prostate (VP), and seminal vesicle (SV). The RNA was separated by electrophoresis and subjected to Northern blot analyses using cDNA probes for MT I **(A)**, MT II **(B)**, and MT III **(C)**. Resulting autoradiograms were quantified by densitometry, and RNA loading was standardized by hybridizing the membranes with oligonucleotide probe recognizing the 18S rRNA **(D)**.

as described above. On the basis of the immunocytochemical results, a second experiment was done with 4 groups of rats: an intact control group and orchidectomy groups with 0, 2.5 cm (mimics blood testosterone levels), and 18.6 cm (epididymal testosterone levels) of testosterone. This was done to determine at the RNA level which MTs were regulated by androgens. Surgery was done as described above, and the rats were sampled 7 days later and their epididymal RNA subjected to Northern blot analyses. The efficiency of the implants was assessed by weighing the epididymides, seminal vesicles, and ventral prostate, which are androgen dependent. At the time of sampling, for both experiments, the integrity of the epididymal vasculature was verified and had remained intact in all cases.

Induction of MT mRNA Levels by Cadmium—Rats were anesthetized with an intraperitoneal injection of ketamine/xylazine (50:10 mg/kg) and given an intravenous injection of $CdCl_2$ (5 μmol/kg) dissolved in physiological saline. Control animals received saline alone. Animals were killed 24 hours after the injection according to the procedure described above. Tissues were processed subsequently for Northern blot analyses.

Northern Blot Analysis

To determine the regional distribution of relative mRNA levels, Northern blot analyses were performed on total cellular RNA isolated from the testis and on each of the 4 epididymal segments. The RNA was isolated according to the guanidinium isothiocyanate method (Chomczynski and Sacchi, 1987), and a 10-μg aliquot of total RNA was separated by electrophoresis in a 1.2% agarose-formaldehyde gel. The RNA was then transferred onto a charged nylon membrane (Genescreen Plus, Dupont Chemicals, Missassauga, Canada), as described by Viger and Robaire (1991).

Two cDNA probes for MT I and MT II were obtained from Dr F. Denizeau (University of Quebec at Montreal; Moffatt et al, 1995), and an MT III probe was obtained by RT-PCR as described previously (Dufresne and Cyr, 1999). The cDNA probes were labeled by random priming with [^{32}P]-dCTP using the Oligonucleotide Labelling Kit (Pharmacia-Amersham Biotech, Baie D'Urfe, Canada). Each Northern blot was standardized for RNA loading by hybridizing the membranes with an oligonucleotide probe that recognized the 18S rRNA (Cyr et al, 1992). Hybridizations of the MT cDNA probes and 18S rRNA probe were done according to the method outlined by Viger and Robaire (1991). The resulting unsaturated autoradiograms were scanned with a Bio-Rad Fluor Image analyzer, and the integrated area under the curve for each signal was standardized against the signal for the 18S rRNA in order to determine the relative concentrations of MT I, MT II, and MT III mRNA (Figure 2A

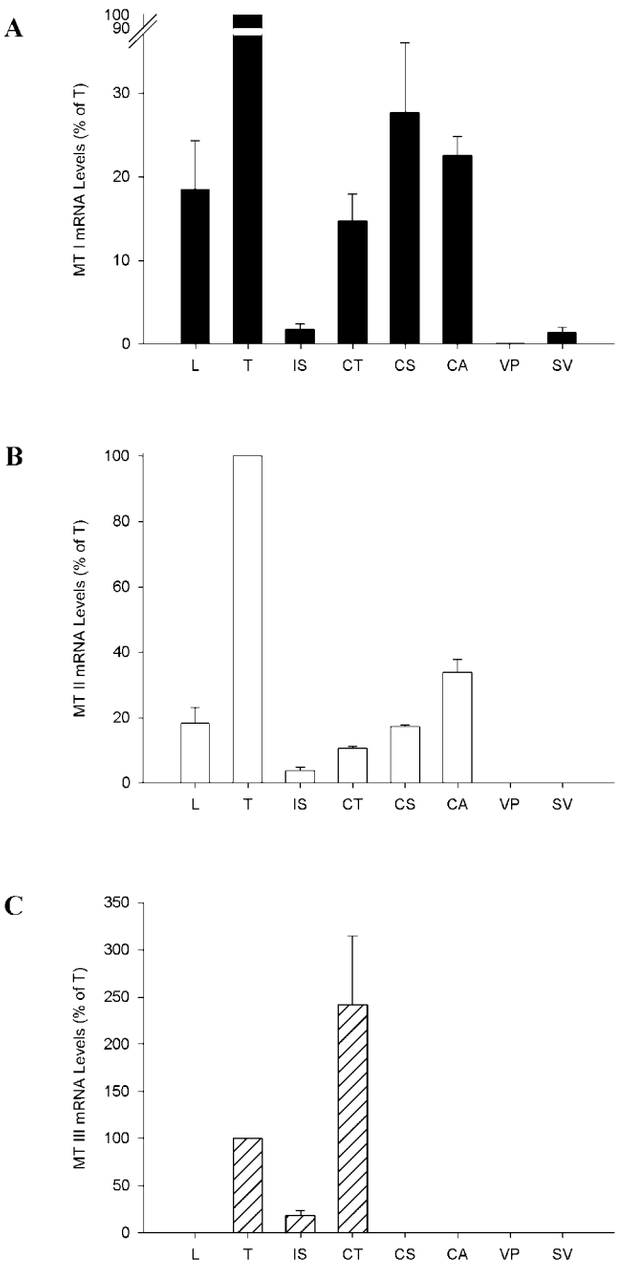

Figure 3. Distribution of MT mRNA along the male reproductive tract of adult rat. Total cellular RNA was isolated from the liver (L indicates positive control), testis (T), epididymal segments (initial segment [IS], caput [CT], corpus [CS], and cauda [CA] epididymidis), ventral prostate (VP), and seminal vesicles (SV). The RNA was separated by electrophoresis and subjected to Northern blot analyses using cDNA probes for MT I **(A)**, MT II **(B)**, and MT III **(C)**. Resulting autoradiograms were quantified by densitometry, and RNA loading was standardized by hybridizing the membranes with oligonucleotide probe recognizing the 18S rRNA. Data are expressed as the mean ± SEM (n = 3 rats).

through D). Four different blots containing RNA from 4 different rats were used to determine the relative mRNA levels in the testis and in each of the epididymal segments.

Statistical Analyses

Normality of the data was determined using the Kolmogorov-Smirov tests; the Levine median test was done for equal variance. Statistical differences between groups were determined by ANOVA, followed a posteriori by a Student-Newman-Keuls test for multiple comparisons between experimental groups. Significance was established at $P < .05$. All analyses were done by the SigmaStat computer software (Jandel Scientific Software, San Rafael, Calif).

Results

MT mRNA Levels Along the Male Reproductive Tract

MT I and MT II mRNA levels in adult rats were most abundant in the testis; levels there were more than 5 times greater than were levels in the liver (positive control; Figure 3A and B). In the epididymis and accessory glands, MT I mRNA levels were highest in the caput, corpus, and cauda epididymidis; those levels were 10–20 times greater than were the levels in the initial segment and seminal vesicles. The lowest MT I mRNA levels were observed in the ventral prostate (Figure 3A). MT II mRNA levels were highest in the testis; those levels were more than threefold higher than in the cauda epididymidis (Figure 3B). In the epididymis, MT II mRNA levels were highest in the cauda; those levels were twofold higher than in the corpus, threefold higher than in the caput, and 10-fold higher than in the initial segment. MT II mRNA levels in the ventral prostate and seminal vesicles were below the level of detection for the assay (Figure 3B). MT III transcripts were detectable only in the testis, initial segment, and caput epididymidis (Figure 3C). MT III mRNA levels were highest in the caput; those levels were 2.5-fold greater than in the testis and 14-fold greater than in the initial segment (Figure 3C).

Immunolocalization of MT

At the light microscope level, there was a complete absence of reaction product over the epithelial cells of the entire epididymis in control tissues treated with normal rabbit antiserum (data not shown). In tissues treated with MT antibody, a distinct immunoperoxidase reaction was observed over the cytoplasm of basal cells of the epididymis (Figure 4A and B). No staining was observed over the nuclei of the basal cells. Interestingly, not all basal cells in any given region were reactive, and there appeared to be a greater number of reactive basal cells in the cauda epididymidis, followed by the levels in the corpus and caput epididymidis (Figure 4A and B). The least reactive basal cells were observed in the initial segment.

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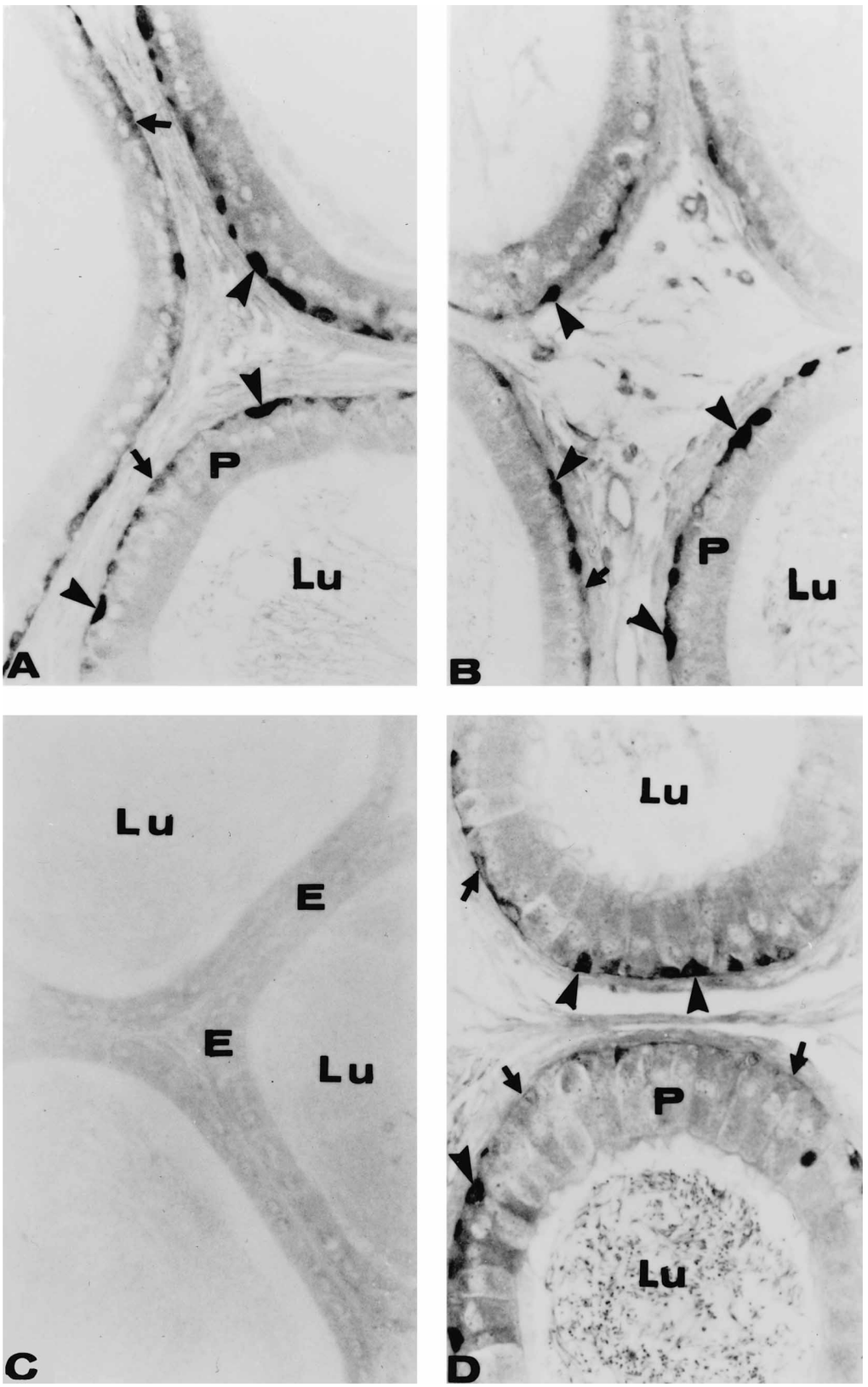

In the cauda epididymidis, immunoreactive staining was also observed over some, but not all, clear cells (not shown). No staining was observed over the epithelial principal cells or over the spermatozoa in the epididymal lumen.

Regulation by Androgens

In epididymides of orchidectomized rats sampled 3, 7, 14, and 21 days after surgery, there was no immunoreactivity over the epithelium throughout the epididymis (only day 14 data is shown). A section of the proximal cauda epididymidis of rats 14 days after surgery is shown as an example of the loss of reaction product (Figure 4C). In orchidectomized rats that received testosterone implants and were sampled 14 days after surgery, MT reaction was noted over the cytoplasm of basal cells; it was similar to the reaction observed in intact control animals in each region of the epididymis (Figure 4D).

In order to determine whether all 3 MTs were regulated in the same manner, an experiment was done in which rats were orchidectomized and given testosterone replacement (0, 2.5, and 18.6 cm; Figure 5). To determine the efficiency of the testosterone implants, the paired weights of the epididymis and seminal vesicles, as well as the ventral prostate weight, were measured. These data indicate that tissue weights for all 3 organs were significantly decreased after orchidectomy (Table 1). Androgen replacement resulted in a dose-dependent increase in tissue weights. In the ventral prostate and seminal vesicles, tissue weights exceeded control levels in the high-testosterone group, which is consistent with the fact that these tissues are normally exposed to the lower blood levels of testosterone (Table 1). In the epididymis, tissue weights in the high-testosterone group were similar to those in controls (Table 1).

Total cellular RNA was isolated from each epididymal segment and subjected to Northern blot analysis using the cDNA probes for MT I, MT II, and MT III. The results indicate that MT I mRNA levels in each of the 4 epididymal segments were significantly lower in orchidectomized rats (Figure 4). In each segment, except the corpus, epididymal testosterone levels (18.6 cm) maintained MT I mRNA levels at control values. In the corpus, high- and low-dose testosterone–implanted rats had higher levels of MT I mRNA than orchidectomized rats, but levels were lower than controls (Figure 5).

MT II mRNA levels in the initial segment were decreased by almost 75% in orchidectomized rats (Figure 6). MT II mRNA levels were almost twofold higher in rats implanted with 2.5 cm of testosterone and were similar to control levels in rats implanted with 18.6 cm of testosterone. MT II mRNA levels appeared to be somewhat less sensitive than MT I in the caput and cauda epididymidis, in which MT II mRNA levels were not significantly different among any of the experimental groups (Figure 6). In the corpus epididymidis, orchidectomy resulted in a sevenfold decrease in MT II mRNA levels. MT II mRNA levels in testosterone-implanted rats, at both doses, were not maintained at control levels (Figure 6).

MT III mRNA levels in the initial segment were unaltered by orchidectomy (Figure 7). In orchidectomized rats given either doses of testosterone, there was a 9- to 11-fold increase in MT III mRNA levels as compared with the case of controls. There were no differences in MT III mRNA levels between the different testosterone doses, suggesting that MT III is maximally expressed by serum testosterone levels in orchidectomized rats. Interestingly, in the caput epididymidis, MT III mRNA levels were sixfold lower in orchidectomized rats, and levels were maintained at control levels with either dose of testosterone.

Induction of MT mRNA Levels by Cadmium

To determine whether or not epididymal MTs can be induced by heavy metals, rats were administered a single intravenous injection of $CdCl_2$ because cadmium and zinc are the best inducers of MTs in both liver and kidney. Rats were killed 24 hours after injection in order to minimize secondary effects resulting from cadmium administration. In the liver, MT I mRNA levels were not significantly higher ($P = .06$) in the cadmium-treated rats as compared with saline-injected controls (Figure 8A). MT II mRNA levels, however, were significantly higher in the livers of cadmium-treated rats (Figure 8B). In the testis, cadmium caused an almost twofold increase in MT I mRNA levels, but there were no differences in MT II and MT III mRNA levels (Figure 7). In the initial segment, MT I and MT II mRNA levels were significantly increased by cadmium treatment, but MT III mRNA levels were not different from controls. In the caput epididymidis, MT II mRNA levels were significantly increased by

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Figure 4. Immunocytochemical localization and regulation of MT in the rat epididymis. In the corpus **(A)** and cauda **(B)** epididymidis of intact adult rats, MT was immunolocalized, mainly over basal cells (arrowheads). In some basal cells, MT immunoreactivity was weak or completely absent (arrows). No reaction was observed over principal cells or spermatozoa in the epididymal lumen. In rats orchidectomized for 14 days, immunoreactive staining was completely absent in all the regions of the epididymis as noted in the cauda **(C)**. In orchidectomized rats implanted with 18.6-cm testosterone implants (epididymal testosterone levels), MT immunostaining over the basal cells was similar to controls as noted in the cauda (D). As in controls, some basal cells showed a strong MT immunoreaction (arrowheads), whereas others were weak or nonreactive (arrows). P indicates principal cells; Lu, lumen; and E, epithelium.

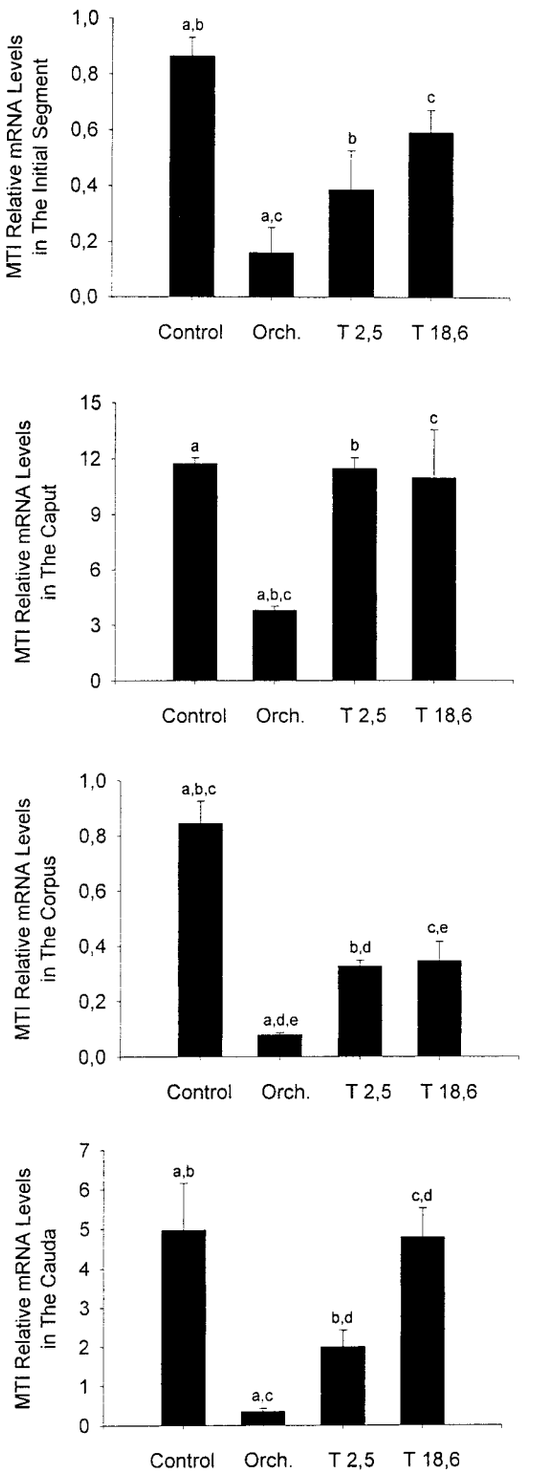

Figure 5. Regulation of MT I mRNA levels by androgens. Northern blot analyses were done on total cellular RNA isolated from each segment of the epididymis (initial segment, caput, corpus, and cauda epididymidis) taken from intact control (Ctrl), orchidectomized (Orch), and orchidectomized rats implanted with either a 2.5-cm (T2.5) or an 18.6-cm (T18.6) testosterone-filled capsule. Data was standardized by hybridizing the blots with an 18S rRNA probe. Data are expressed as the mean ± SEM (n = 4 rats per group). Groups with the same letter indicate a statistical difference ($P \leq .05$).

almost fourfold. A similar increase was observed in MT I mRNA levels, although this was not statistically different ($P = .06$). There were no differences in MT III mRNA levels. In the corpus, MT II mRNA levels were significantly decreased by cadmium exposure, whereas there were no statistical differences in MT I mRNA levels. In the cauda epididymidis, MT I and MT II mRNA levels were unaltered by cadmium treatment (Figure 8A and B). There were no differences in MT I and MT II mRNA levels in the seminal vesicles and ventral prostate between control and cadmium-injected rats.

Discussion

In the present study, immunoreactive MT was distributed in basal cells throughout the epididymis; however, not all basal cells were reactive in any given region. Furthermore, more cells appeared to be stained in the cauda region, followed by the corpus, caput, and initial segment (data not shown). A reaction over basal cells has also been reported in the human epididymis (Nishimura et al, 1990; Suzuki et al, 1992). Interestingly, previous studies have indicated that basal cells contain various isoforms of glutathione-*S*-transferase (GST; Papp et al, 1995). Furthermore, similar to the case with MT staining, the various isoforms of GST, with the exception of the Y_c-subunit, were noted in basal cells of the corpus and cauda epididymidis, where sperm are stored. However, unlike the case with MT staining, the GST subunits were in all basal cells of the region in which they were expressed (Papp et al, 1995). In addition, the GST isoforms also stained principal cells, which was not the case for MT. Because both GSTs and MTs can deactivate a variety of electrophiles, including free oxygen radicals, basal cells may be responsible for protecting either the epithelia or maturing spermatozoa from electrophilic attack by oxygen free radicals (Andrews, 2000). It has long been established that the sperm membrane is very sensitive to a variety of reactive oxygen species (Alvarez et al, 1987; Tramer et al, 1998). Although there are protective antioxidative enzymes present in semen that protect sperm from free oxygen radicals (Jeulin et al, 1989; de Lamirande et al, 1998; Tramer et al, 1998), it is possible that one of the functions of epididymal basal cells is to protect either the maturing spermatozoa or epididymal principal cells from reactive oxygen species by limiting their entry via the circulation. Studies have demonstrated that basal cells possess long, thin processes that extend around the periphery of tubules, thus forming an incomplete barrier (Veri et al, 1993). However, because MT I and MT II knockout animals remain fertile, it may be suggested that there are compensatory mechanisms for MT function in the epididymis (Klaassen and Liu, 1998).

Table 1. *Effects of orchidectomy and orchidectomy with testosterone replacement on body, paired epididymal, seminal vesicle, and ventral prostate weights at 7 days after surgery**

Experimental Group†	Body Weight (g)	Paired Epididymal Weight (g)	Paired Seminal Vesicle Weight (g)	Ventral Prostate Weight (g)
Control	311 ± 6	0.76 ± 0.04‡	0.35 ± 0.01‡	0.48 ± 0.09‡,§
Orch + 0 T	303 ± 46	0.41 ± 0.12‡,§,‖	0.17 ± 0.06‡,§,‖	0.07 ± 0.12‡,‖,¶
Orch + 2.5 T	315 ± 3	0.66 ± 0.01§	0.31 ± 0.03§,¶	0.30 ± 0.02§,‖,#
Orch 18.6 T	298 ± 48	0.76 ± 0.13‖	0.62 ± 0.11‡,‖,¶	0.58 ± 0.10¶,#

‡,§,‖,¶,# Groups with the same designator indicate statistical difference ($P \leq .05$).

* Data are expressed as mean ± SEM; n = 4 rats per group.

† Orch + 0 T indicates orchidectomy; Orch + 2.5 T, orchidectomy with 2.5-cm testosterone implant; Orch + 18.6 T, orchidectomy with 18.6-cm testosterone implant.

Northern blot analyses of RNA isolated in different organs along the male reproductive tract revealed that MT I and MT II mRNA levels were highest in the testis, followed by the distal regions of the epididymis. MT I and MT II mRNA levels in the ventral prostate and seminal vesicles were at the level of detection. The relatively elevated levels of MT I and MT II mRNA, as well as the apparent higher levels of MT protein in the cauda epididymidis, further suggests that these proteins may be involved in protecting fully matured spermatozoa while they are stored in the cauda epididymidis. MT III mRNA levels are highest in the caput epididymidis; those levels are twofold higher than those in the testis. Unlike MT I and MT II, MT III has been implicated in regulating zinc homeostasis in motor neurons, as opposed to protecting cells from heavy metal exposure or from scavenging free oxygen radicals (Masters et al, 1994). The role of MT III in the testis and epididymis remains to be elucidated, particularly because our antisera recognizes only MT I and MT II, and thus, the localization of MT III in the epididymis still needs to be established.

The loss of immunoreactive MT (MT I and MT II) in basal cells of orchidectomized rats (Figure 4C) and its maintenance in the presence of testosterone (Figure 4D) suggests that MT I and MT II protein levels are dependent on androgens. Although several studies have reported on the regulation of epididymal genes in the epithelial principal cells that line the lumen (Cyr and Robaire, 1992; Palladino et al, 1994; Cyr et al, 1996; Kirchoff, 1999), to our knowledge, there have not been any studies to indicate that androgens act directly on basal cell function. In fact, previous studies on the Y_f-subunit of GST have reported that orchidectomy does not result in any change in the immunostaining of the epididymal basal cells of the rat (Hermo and Papp, 1996). Therefore, the lack of immunoreactive MT in basal cells after orchidectomy and its maintenance by testosterone suggests that basal cell expression of MT is regulated by androgens. Whether or not androgens regulate MT in basal cells via direct or indirect mechanisms remains to be demonstrated.

Previous studies have reported that MT III mRNA levels in ventral prostate can be regulated by androgens (Moffatt and Seguin, 1998). Results in the present study indicate that in the epididymis, mRNA levels for all 3 MTs are regulated by androgens. This regulation, however, is segment specific, and the sensitivity to androgens differs between MT I and MT II. In the caput and cauda epididymidis, MT I mRNA levels (70%–90% lower; Figure 5) were substantially more sensitive to orchidectomy than were MT II mRNA levels, which were not significantly different from those of controls (Figure 6). Differences in the regulation of MT I and MT II mRNA have previously been reported in both testis and epididymis (McKenna et al, 1996; Dufresne and Cyr, 1999). In the present study, the lower sensitivity of MT II mRNA levels to androgens in the caput and cauda epididymidis does not seem to occur at the protein level because immunoreactive MT disappears after orchidectomy.

In the corpus epididymidis, MT I and MT II mRNA levels could not be maintained by testosterone replacement, suggesting that other testicular factors are involved in the regulation of these genes in this segment. Segment-specific regulation by nonandrogenic testicular factors has been previously reported for a number of different genes and is likely the case in the corpus for these 2 genes (Cornwal and Hann, 1995; Kirchoff, 1999).

The regulation of MT III in the initial segment of the epididymis appears to be quite different from the regulation of MT I and MT II in the other epididymal segments. In this region, MT III mRNA levels were unaltered by orchidectomy but were significantly higher in testosterone-replaced, orchidectomized rats relative to intact control animals. This suggests that a factor of testicular origin may repress the androgen regulation of MT III in the initial segment of the epididymis. In the absence of such a factor, as would occur in orchidectomized rats, MT III mRNA levels become highly sensitive to androgen stimulation. To our knowledge, this type of regulation has not previously been observed in the epididymis.

To determine whether epididymal MT mRNA levels were inducible by heavy metals, we administered $CdCl_2$ and measured MT mRNA levels 24 hours after intrave-

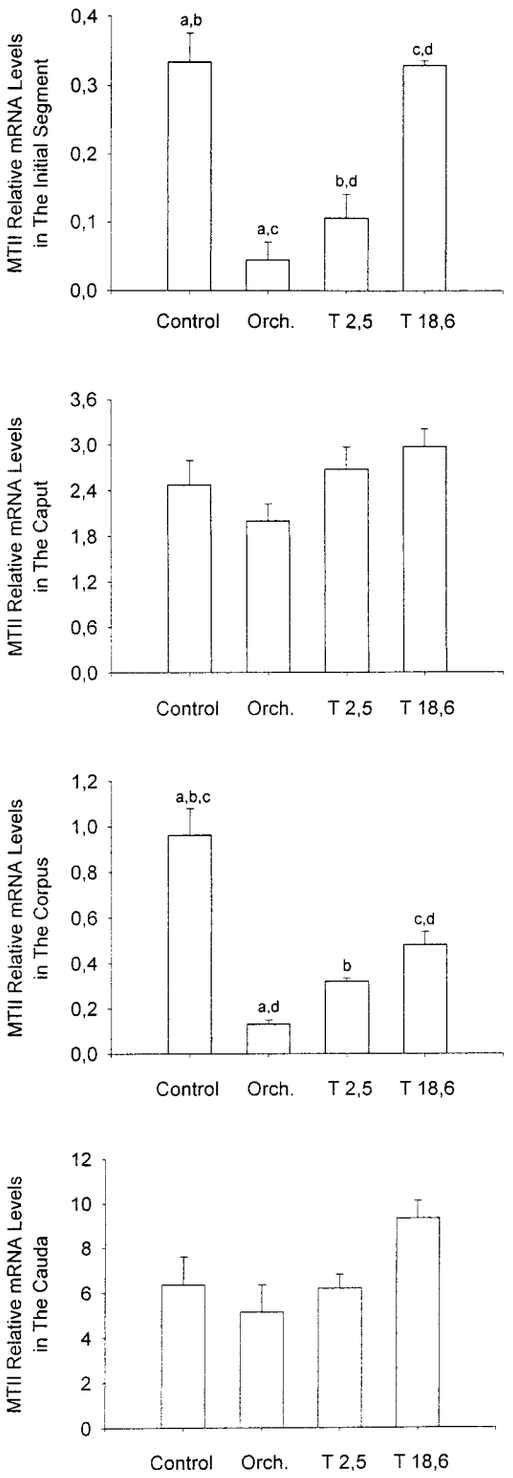

Figure 6. Regulation of MT II mRNA levels by androgens. Northern blot analyses were done on total cellular RNA isolated from each segment of the epididymis (initial segment, caput, corpus, and cauda epididymidis) removed from intact control (Ctrl), orchidectomized (Orch), and orchidectomized rats implanted with either a 2.5-cm (T2.5) or an 18.6-cm (T18.6) testosterone-filled capsule. Data were standardized by hybridizing the blots with an 18S rRNA probe. Data are expressed as the mean ± SEM (n = 4 rats per group). Groups with the same letter indicate a statistical difference ($P \leq .05$).

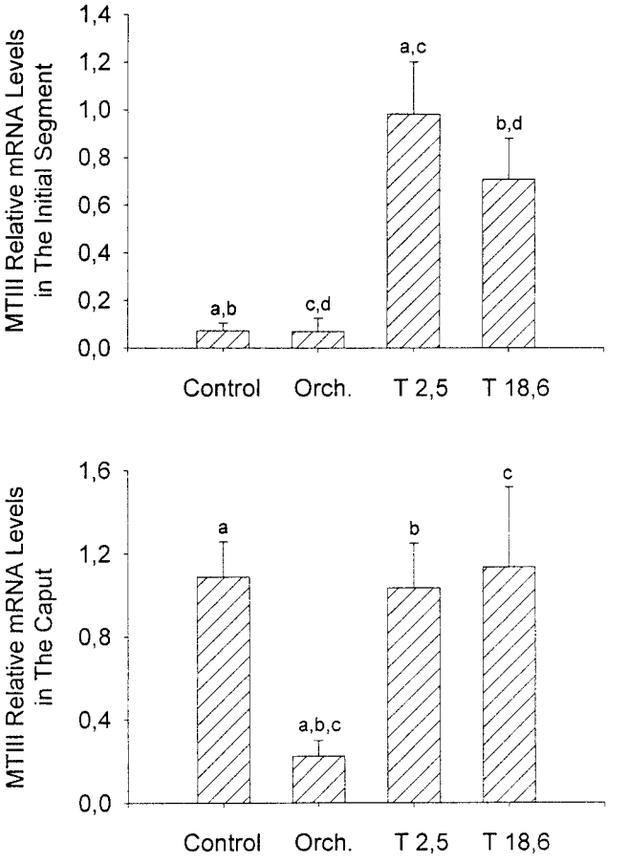

Figure 7. Regulation of MT III mRNA levels by androgens. Northern blot analyses were done on total cellular RNA isolated from each segment of the epididymis (initial segment, caput, corpus, and cauda epididymidis) removed from intact control (Ctrl), orchidectomized (Orch), and orchidectomized rats implanted with either a 2.5-cm (T2.5) or an 18.6-cm (T18.6) testosterone-filled capsule. Data were standardized by hybridizing the blots with an 18S rRNA probe. Data are expressed as the mean ± SEM (n = 4 rats per group). Groups with the same letter indicate a statistical difference ($P \leq .05$).

nous cadmium exposure. In this manner, it was possible to test the rapid response of the different regions of the epididymis to heavy metals. We chose cadmium as a representative heavy metal because it has been shown to be the best inducer of MT in the liver and kidney (Klaassen et al, 1999). The dose selected is considered a low dose and has been reported not to cause testicular lesions (McKenna et al, 1996). Our present observations on the induction of MT I and MT II mRNA in the testis are similar to those reported by McKenna et al (1996), who injected rats with 4 μmol/kg and took samples from them 24 hours later. Those investigators reported a 5.3-fold increase in testicular MT I mRNA levels, with no effect on MT II mRNA levels.

Studies have shown that the prostate and testis are relatively insensitive to heavy-metal stimulation (De et al, 1991; Waalkes and Rehm, 1994). In general, this also

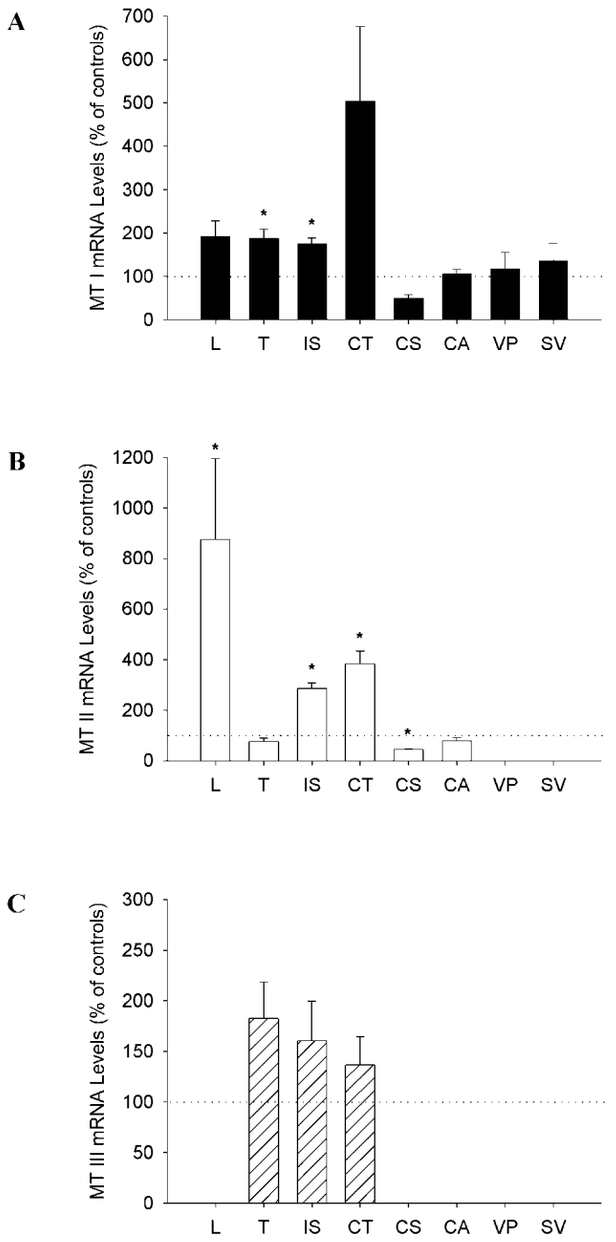

Figure 8. Induction of MT I **(A)**, II **(B)**, and III **(C)** mRNA levels by Cd. Rats were administered an intravenal injection of either saline (controls) or $CdCl_2$ (5 μmol/kg). Rats were sampled 24 hours later. Northern blot analyses were done on total cellular RNA isolated from the liver (L), testis (T), 4 epididymal segments (initial segment [IS], caput [CT], corpus [CS], and cauda [CA] epididymidis), ventral prostate (VP), and seminal vesicles (SV). Data were standardized by hybridizing the blots with an 18S rRNA probe. Data are presented relative to control levels (dotted line) to enable comparisons between tissues and are expressed as the mean ± SEM (n = 5 rats per group). Asterisks denote a statistical difference ($P \leq .05$) between control and cadmium-treated rats.

appears to be the case for the epididymis. The exception may be the caput region of the epididymis, which, along with liver, was the most sensitive to cadmium treatment with respect to increased MT I and MT II mRNA levels. Interestingly, in the caput epididymidis of control rats, immunoreactive MT levels were low, as were MT I and MT II mRNA levels. The differences in cadmium sensitivity along the epididymis are difficult to explain. One possibility is that the transport of cadmium to each epididymal segment may vary, thereby explaining the differences in induction. A recent study by King et al (1999) has reported differences in transport of ^{109}Cd in tissues with cellular barriers (eg, testis and epididymis) between 2 different strains of mice that show differences in sensitivity to cadmium. This difference appears to result from the presence of a saturable cadmium cellular transporter in the most sensitive strain. The presence of a specific cadmium transporter could explain differences in cadmium transport in the testis and along the epididymis, depending on the distribution of the transporter in the different regions of the epididymis. The presence of a transport system in the testis and epididymis is further supported by the observation that the epididymis of immature rats is refractive to cadmium before the formation of the blood-epididymal barrier (Sacerdote and Cavicchia, 1995). Once the barrier is formed, however, cadmium exposure can alter cell-cell interaction and intercellular junctions between adjacent principal cells (Sacerdote and Cavicchia, 1995).

In a previous study, we reported that the administration of MeHg to adult rats resulted in differences in MT mRNA levels in the different segments of the epididymis. This study suggested either that MeHg transport to the different regions of the epididymis varied or that the sensitivity of the segments to MeHg is not uniform (Dufresne and Cyr, 1999). As seen in the MeHg study, cadmium exposure resulted in increased MT I and MT II mRNA levels in the proximal regions of the epididymis, whereas levels in the corpus and cauda epididymidis (distal epididymis) were either unaffected or decreased. Although both chemicals act very differently at the level of the cell, the similarities in results for both studies suggest that there may be common features with respect to metal detoxification along the epididymis or with the absorption of metals and access to cells. Furthermore, because MT I and MT II expression is low in the proximal segments of the epididymis, these may be more likely to respond to a challenge by heavy metals, as compared with the case of the cauda epididymidis, in which MT levels are elevated and therefore may be less sensitive to heavy metals. In the distal segments, metals may be rapidly sequestered by MTs as they enter the cell and, therefore, be unavailable to stimulate MT transcription.

In summary, the results of the present study demonstrate that MT is present mainly in the basal cells of the rat epididymis and that although mRNA levels in the epididymis are lower than those in testis, they are generally higher than levels found in either the ventral prostate or

seminal vesicles. Both mRNA levels for all 3 MTs and immunoreactive MT protein are regulated by testicular androgens. Although the regulation of MTs by testosterone is not segment specific, cadmium-induced MT stimulation occurred only in the initial segment and caput epididymidis, suggesting that these differences may be related to differences in preferential accumulation of cadmium in the different regions of the epididymis or that these regions have the least number of reactive basal cells.

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