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Effect of stress, adrenalectomy and changes in glutathione metabolism on rat kidney metallothionein content: comparison with liver metallothionein

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Eighteen hours of immobilization stress, accompanied by food and water deprivation, increased liver metallothionein (MT) but decreased kidney MT levels. Food and water deprivation alone had a significant effect only on liver MT levels. In contrast, stress and food and water deprivation increased both liver and kidney lipid peroxidation levels, indicating that the relationship between MT and lipid peroxidation levels (an index of free radical production) is unclear. Adrenalectomy increased both liver and kidney MT levels in basal conditions, whereas the administration of corticosterone in the drinking water completely reversed the effect of adrenalectomy, indicating an inhibitory role of glucocorticoids on MT regulation in both tissues. Changes in glutathione (GSH) metabolism produced significant effects on kidney MT levels. Thus, the administration of buthionine sulfoximine, an inhibitor of GSH synthesis, decreased kidney GSH and increased kidney MT content, suggesting that increased cysteine pools because of decreased GSH synthesis might increase kidney MT levels through an undetermined mechanism as it appears to be the case in the liver. However, attempts to increase kidney MT levels by the administration of cysteine or GSH were unsuccesful, in contrast to what is known for the liver. The present results suggest that there are similarities but also substantial differences between liver and kidney MT regulation in these experimental conditions.

Keywords: glucocorticoids, glutathione, metallothionein, stress

Introduction

Metallothionein (MT), a low molecular weight, cysteine-rich, heavy metal-binding protein, has been involved in zinc and copper metabolism and in heavy metal detoxification (Cousins 1985, Bremner 1987, Kägi & Kojima 1987, Klaassen & Lehman-McKeeman 1989). Most of what is known of this protein is related to the liver and the kidney, but its actual physiological function(s) remains uncertain. The induction of liver MT by several stress factors is well-known (Oh *et al.* 1978, Sobocinski *et al.* 1978, Brady 1981, Hidalgo *et al.* 1988a, b); the role of liver MT during stress is not precisely known, although it has been suggested that MT might function in protection against free radicals (Thornalley & Vasak 1985, Thomas *et al.* 1986; Hidalgo *et al.* 1988a). Although the kidney is one of the tissues more important with regard to some of the putative MT functions, it is surprising that very few efforts have been made to establish the influence of stress on kidney MT levels. Early studies indicate little, if any, effect of stress on kidney MT (Oh *et al.* 1978), but the fact that brain and heart in addition to liver MT levels are significantly increased by immobilization stress (Hidalgo *et al.* 1990a) indicates the need for more studies on the effect of stress on kidney MT.

On the other hand, we have recently shown that MT is somehow related to glutathione (GSH) metabolism in the liver (Hidalgo *et al.* 1990b); in the present report we evaluate the putative relationship of MT and GSH in the kidney by inhibiting GSH synthesis or degradation.

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Materials and methods

Animals

Adult male Sprague-Dawley rats were used. Rats were housed in groups of four per cage, under standard conditions (lights on 07:00–19:00 h, temperature 22 °C, food and water *ad libitum*) for at least 1 week before starting experiments.

Materials

All chemicals were reagent grade. Corticosterone, glutathione, L-buthionine-(*S*, *R*)-sulfoximine and AT-125 were purchased from Sigma (St Louis, MO, USA). 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB) was purchased from Fluka AG (Buchs, Switzerland). Laboratory food was from Panlab (Barcelona, Spain). Rats were from the breeding facility of the University.

Experiment 1: effect of starvation and immobilization stress

Rats (350 ± 35 g body weight) were subjected to 2, 4, 8 and 18 h of immobilization stress (Repcekova & Mikulaj 1977). For immobilization, the animal's rear and forelimbs were taped to metal mounts fixed to a board; head motion was limited by metal loops fixed over the neck area. As stressed rats have no access to either food or water, other rats were subjected to food and water deprivation for 4, 8 and 18 h. Another group of rats that were maintained without any disturbance was killed at the same time as 18 h stressed or starved rats.

Experiment 2: effect of adrenalectomy and corticosterone therapy

Rats (280 ± 30 g body weight) were bilaterally adrenalectomized (ADX) or sham-ADX under ether anesthesia. The rats were housed two per cage. ADX rats were randomly assigned to two experimental groups: (i) ADX, rats provided with saline to drink, and (ii) ADX + corticosterone, rats provided with saline containing 100 $\mu g\,ml^{-1}$ corticosterone. Sham-ADX rats were given tap water. The hormone solution was freshly prepared daily. The rats were killed 6 days later.

Experiment 3: inhibition of GSH synthesis by buthionine sulfoximine (BSO)

Rats (279 ± 50 g body weight were treated twice (at 10 and 18 h) with BSO (2 $mmol\,kg^{-1}$) or saline (NaCl) and were killed 24 h after the first injection. Other rats were treated in the same manner, but for 2 days, and were killed 48 h after the first injection.

Experiment 4: effect of GSH and AT-125 administration

Rats (242 ± 14 g body weight were assigned to four experimental groups: (i) NaCl, rats that were treated only with saline and killed at the same time as the other groups; (ii) AT-125, rats that were treated (i.p.) with the irreversible inhibitor of gamma glutamyl transpeptidase (GGT), AT-125 (15 $mg\,kg^{-1}$), at 10 h and with saline at 11, 15 and 19 h, and that were killed 24 h after the AT-125 injection; (iii) GSH, rats that were treated (i.p.) with saline at 10 h and with GSH (100 $mg\,kg^{-1}$) at 11, 15 and 19 h, and that were killed 24 h after the saline injection; and (iv) AT-125 + GSH, rats that were treated (i.p.) with AT-125 (15 $mg\,kg^{-1}$) at 10 h and with GSH (100 $mg\,kg^{-1}$) at 11, 15 and 19 h, and that were killed 24 h after the AT-125 injection.

Experiment 5: effect of cysteine administration

The rats (410 ± 43 g body weight) were randomly assigned to four experimental groups: (i) NaCl, rats that were injected daily (i.p.) with saline for 4 days; (ii) GSH, rats that were injected daily (i.p.) with GSH (100 $mg\,kg^{-1}$, about 0.325 $mmol\,kg^{-1}$) for 4 days; (iii) cysteine 1, rats that were injected daily (i.p.) with cysteine (25 $mg\,kg^{-1}$, about 0.206 $mmol\,kg^{-1}$) for 4 days; and (iv) cysteine 2, rats that were injected daily (i.p.) with cysteine (50 $mg\,kg^{-1}$, about 0.412 $mmol\,kg^{-1}$) for 4 days. Some rats received a single cysteine injection (50 $mg\,kg^{-1}$). All rats were killed 24 h after the last injection.

Assays

Rats were killed by decapitation in a room adjacent to the animal quarters. Kidney and liver were immediately removed and frozen at −85 °C in a Revco ultrafreezer. After their thawing liver and kidney were homogenized (2 and 3 v/w, respectively) in ice-cold 10 mM Tris–HCl, pH 8.2, containing 0.25 M sucrose, 2 mM 2-mercaptoethanol, 10 mM sodium azide and 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and centrifuged at 50000 × *g* (30 min, 4°C) in a Kontron Centrikon H-401 high-speed centrifugue. The supernatants were stored in aliquots at −20 °C until the assays were done. Tissue MT levels were measured by a cadmium-saturation method as detailed elsewhere (Hidalgo *et al.* 1988b), measuring also copper in the samples since cadmium cannot displace the former from MT. In Experiment 2, MT levels were measured by a radioimmunoassay (RIA) developed in our laboratory; briefly, polyclonal antibodies were raised in rabbits against rat Cd7-MT2 that were used in a double antibody RIA. The assay was performed using the following components and volumes in each tube assay: 300 μl of Tris–gelatine buffer (50 mM Tris–HCl, 0.1% gelatine, 0.1% sodium azide, pH 8.0), 100 μl of unknown or standard and 50 μl of first antibody solution, which contains 0.05 M EDTA, 2.5% of normal rabbit serum and 1:640 dilution of rabbit antiserum (final antibody dilution used in the assay tubes was 1:6400), and then the assay tubes were incubated for 48 h at 4 °C. The tracer was then added (50 μl of iodinated MT-2, about 200 pg of MT or 15000 c.p.m.) and further incubated at 4 °C. The second antibody solution (100 μl of 1:9 diluted goat anti-rabbit antibody in Tris–gelatine buffer) was added 8 h later and the tubes were incubated overnight at 4 °C. The assay tubes were then centrifuged at

4000 r.p.m. for 60 min, the supernatants aspirated and the pellets counted in a gamma counter. Typical standard curves were plotted using Logit-log data transformation where Q is log MT competitor (in pg) and $Z = \log (100Y/(1 - Y))$, where Y is the fraction of antigen bound. The antibody cross-reacted equally with the two MT isoforms (see Figure 3). Lipid peroxidation was assessed by measuring malondialdehyde formation, using the thiobarbituric acid (TBA) assay (Uchiyama & Mihara 1978). Approximate kidney GSH levels were assessed by measuring total non-protein, acid-soluble thiol content by reaction with DTNB (Ellman 1959) since most of the cytosolic thiol content is GSH (Meister 1988); actual kidney GSH levels could not be measured because kidneys were not homogenized in acid immediately after their dissection (because they had to be used for MT measurements), which is necessary to obtain reliable measurements of GSH given the high content of GGT in the kidney (Anderson 1987). A Kontron Uvikon 810 double-beam spectrophotometer was used in the above techniques. Zinc, copper and cadmium levels were measured by atomic absorption spectrophotometry with a Perkin-Elmer 370-A spectrophotometer. All samples to be statistically compared were processed in the same assay to avoid inter-assay variations.

Statistical analysis

When two means were compared Student's t-test or the Mann–Whitney U-test were used. When more than two means were compared one- or two-way analysis of variance (ANOVA) was used. Appropriate individual comparisons between means were done with the Student–Newman–Keuls and Duncan's test (set at $\alpha = 0.05$). Data were subjected to logarithmic transformation where necessary to achieve homogeneity of variances.

Results

Experiment 1: effect of starvation and immobilization stress

Figure 1 shows the effect of food and water deprivation (starvation) alone or accompanied by immobilization stress on liver MT and kidney MT. As expected, liver MT levels were increased by 18 h of starvation ($P < 0.05$), and strongly increased by immobilization stress in a time-dependent manner ($P < 0.001$). In contrast, kidney MT levels were significantly decreased after 18 h of stress ($P < 0.05$).

Figure 2 shows liver and kidney lipid peroxidation levels, which were increased significantly ($P < 0.05$) by 18 h of starvation or stress.

Table 1 shows serum zinc and liver and kidney cytosolic zinc levels. Serum zinc levels were significantly decreased by stress in a time-dependent manner ($P < 0.001$). Liver cytosolic zinc levels were

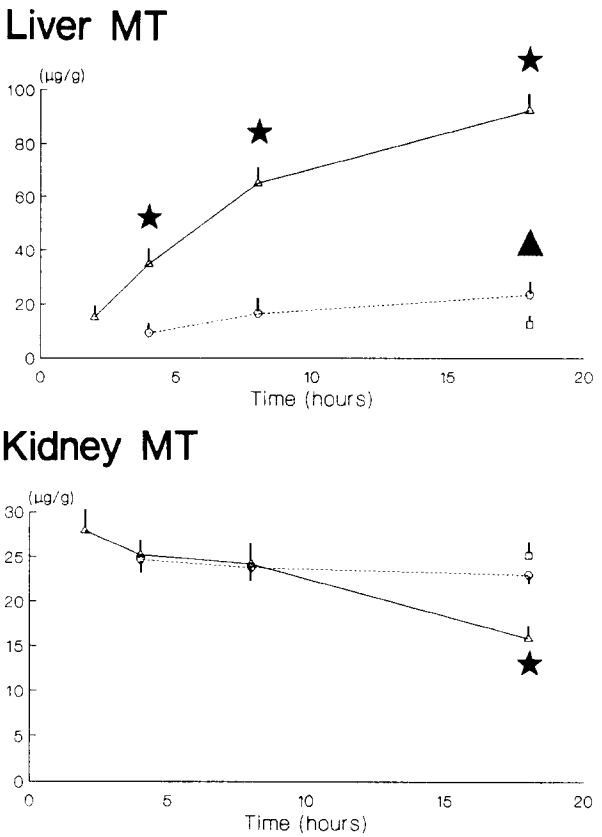

Figure 1. Effect of food and water deprivation alone or accompanied by immobilization stress on liver and kidney MT levels. Values are means ± SE ($n = 5$). Rats were killed after no disturbance (control, □), 4, 8 or 18 h of food and water deprivation (starvation, ○), or 2, 4, 8 or 18 h of food and water deprivation plus immobilization stress (stress, △). Results were analyzed with one-way or two-way ANOVA. When significant (see Results), the differences between appropriate groups were analyzed with the Student's t-test or the Duncan procedure. * P at least below 0.05 versus control rats. ▲ $P < 0.05$ versus control and stressed rats.

increased by starvation and further by immobilization stress in a time-dependent manner ($P < 0.001$). In contrast, kidney cytosolic zinc levels were not significantly altered by either starvation or stress.

Experiment 2: effect of adrenalectomy and corticosterone therapy

Figure 3 shows typical standard curves for the two rat MT isoforms, MT-I and MT-II, obtained with the RIA developed in our laboratory and used for measuring MT levels of this experiment. The polyclonal antibodies cross-reacted equally with the two isoforms. Serial dilutions of tissues paralleled the response of the standard curve (not shown).

Figure 4 shows the effect of adrenalectomy and

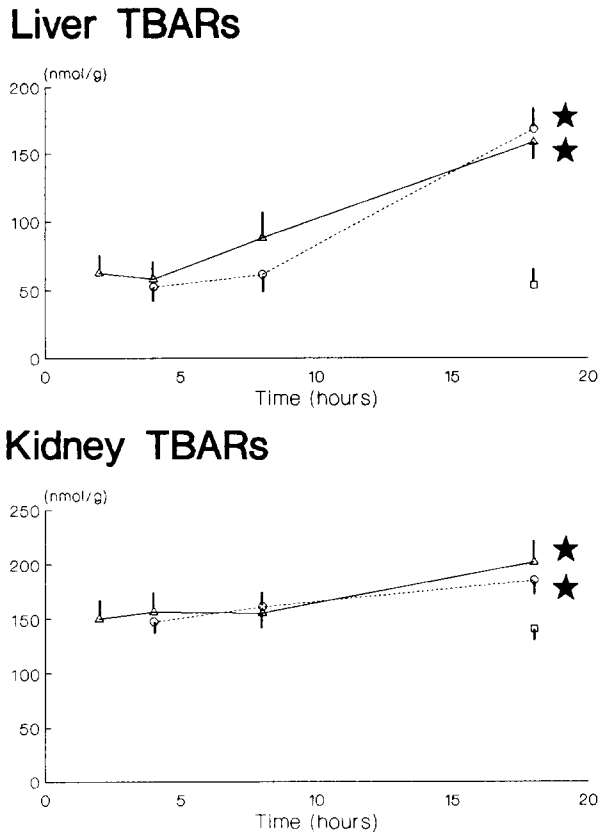

Figure 2. Effect of food and water deprivation alone or accompanied by immobilization stress on liver and kidney lipid peroxidation levels as measured by thiobarbituric acid reactivity (TBARs). For other details, see legend to Figure 1. * P versus control rats.

corticosterone therapy in the drinking water on liver and kidney MT levels, as measured 6 days after the surgical procedure. As expected, liver MT levels were increased by ADX ($P < 0.05$), an effect reversed by corticosterone ($P < 0.05$). The same results were obtained for kidney MT ($P < 0.05$ in both cases).

Experiment 3: inhibition of GSH synthesis by BSO

Figure 5 shows kidney MT and GSH. The administration of BSO increased MT ($P < 0.05$) and decreased GSH levels ($P < 0.05$) after 1 or 2 days of treatment.

Table 2 shows kidney cytosolic zinc and lipid peroxidation. The inhibition of the synthesis of GSH by BSO increased the lipid peroxidation in the kidney ($P < 0.05$) after 2 days of treatment. The cytosolic zinc was slightly increased by the administration of BSO after 1 day of treatment ($P < 0.05$), but after 2 days the difference did not reach statistical significance.

Table 1. Effect of food and water deprivation (starvation) alone or accompanied by immobilization stress on serum zinc and liver and kidney cytosolic zinc.

	Serum zinc ($\mu g\,ml^{-1}$)	Liver zinc ($\mu g\,g^{-1}$)	Kidney zinc ($\mu g\,g^{-1}$)
2 h			
stress	1.86 ± 0.1	22.5 ± 1.7	14.7 ± 0.8
4 h			
starvation	1.97 ± 0.2	21.3 ± 0.5	13.3 ± 0.9
stress	1.53 ± 0.1	25.5 ± 1.1[a]	14.9 ± 1.3
8 h			
starvation	1.87 ± 0.1	25.2 ± 0.7	16.5 ± 1.0
stress	1.00 ± 0.1[a]	30.4 ± 0.5[a]	17.4 ± 0.3
18 h			
control	1.77 ± 0.1	20.6 ± 1.2	13.7 ± 0.3
starvation	1.76 ± 0.1	22.9 ± 1.0	14.4 ± 0.5
stress	1.28 ± 0.2[a]	32.4 ± 1.4[a]	13.1 ± 0.5

Values are mean ± SE ($n = 5$). Rats were subjected to 2, 4, 8 and 18 h of immobilization stress; other rats were subjected to food and water deprivation for 4, 8 and 18 h. Some rats remained in the animal room without any disturbance until they were killed (control). Results were analyzed with one- or two-way ANOVA. When significant (see Results), the differences between appropriate groups were analyzed with Student's t-test or the Duncan procedure.

[a] P at least below 0.05 versus control or starved rats.

Experiment 4: effect of GSH and AT-125 administration

Table 3 shows the MT, GSH, cytosolic zinc and lipid peroxidation in the kidney. The administration of GSH and AT-125, alone or combined, did not affect kidney MT, GSH, cytosolic zinc or lipid peroxidation compared with controls. However, the MT

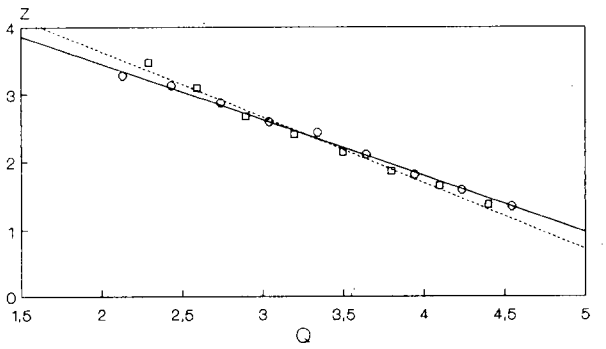

Figure 3. Logit-log regressions for radioimmunoassay ussing MT-1 (□) and MT-2 (○) inhibitory curves with standards ranging from 100 to 50000 pg per tube. The two isoforms show identical behavior.

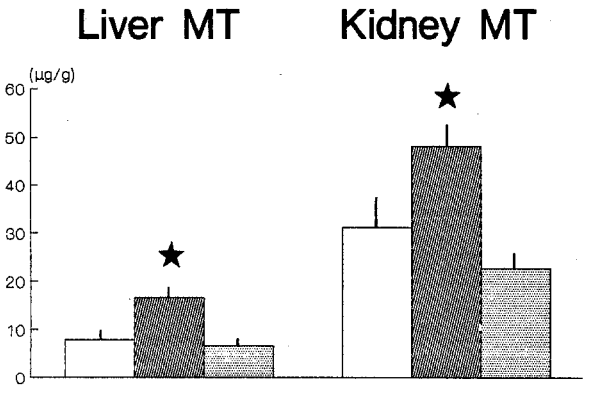

Figure 4. Effect of adrenalectomy and corticosterone therapy on liver and kidney MT. Values are means ± SE (n = 6–7). Rats were either adrenalectomized (ADX) or sham-ADX. Steroid treatment was started the day of surgery, supplying it in the drinking fluid (100 $\mu g\,ml^{-1}$ corticosterone). Rats were killed 6 days after surgery. *$P < 0.05$ versus sham-ADX rats. □, sham; ▨, ADX; □, ADX + corticosterone.

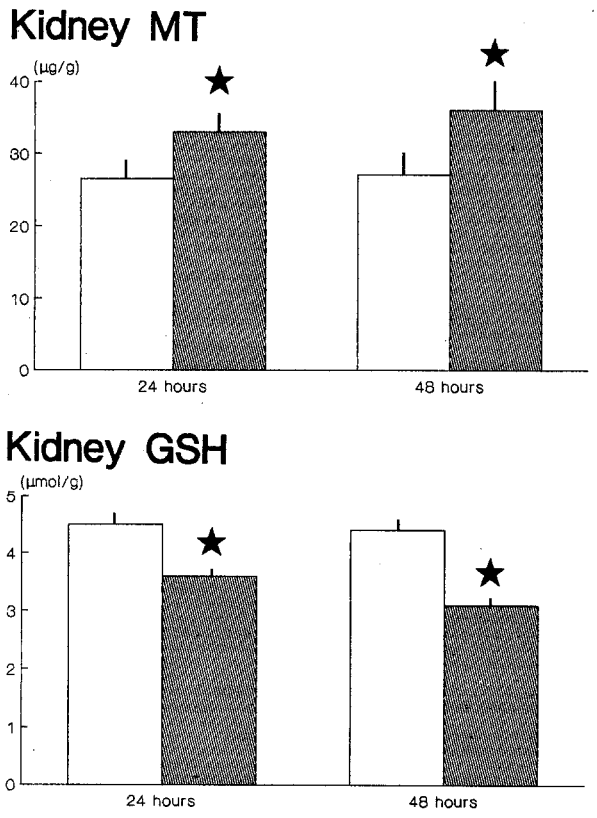

Figure 5. Effect of BSO treatment on kidney MT and kidney non-protein total thiols (GSH) in rats. Values are means ± SE (n = 7–8). Some rats were treated twice (at 10 and 18 h) with BSO (2 mmol kg^{-1}) or saline (NaCl) and were killed 24 h after the first injection. Other rats were treated in the same manner, but for 2 days, and were killed 48 h after the first injection. * $P < 0.05$ versus NaCl rats. □, Saline; ▩, BSO.

Table 2. Effect of BSO on kidney cytosolic zinc and TBA reactants (lipid peroxidation)

	Zinc ($\mu g\,g^{-1}$)	Lipid peroxidation (nmol g^{-1})
24 h		
NaCl	12.8 ± 0.3	136.9 ± 5.6
BSO	14.2 ± 0.4[a]	128.6 ± 8.7
48 h		
NaCl	15.7 ± 1.0	140.1 ± 3.0
BSO	16.1 ± 0.8	164.6 ± 5.5[a]

Values are means ± SE (n = 7–8). Some rats were treated twice (at 10 and 18 h) with BSO (2 mmol kg^{-1}) or saline (NaCl) and were killed 24 h after the first injection. Other rats were treated in the same manner, but for 2 days, and were killed 48 h after the first injection.
[a] $P < 0.05$ versus respective NaCl rats.

Table 3. Effect of glutathione and AT-125, alone and combined, on kidney MT, non-protein thiols (GSH), cytosolic zinc and lipid peroxidation

	MT ($\mu g\,g^{-1}$)	GSH ($\mu mol\,g^{-1}$)	Zinc ($\mu g\,g^{-1}$)	Lipid peroxidation (nmol g^{-1})
NaCl	37.8 ± 3.1	3.60 ± 0.1	12.45 ± 0.5	161.0 ± 10.2
AT-125	46.3 ± 3.1[a]	4.15 ± 0.2	12.92 ± 1.1	164.5 ±8.8
GSH	32.4 ± 2.3	3.85 ± 0.1	13.88 ± 0.4	156.6 ± 8.7
GSH +AT-125	38.6 ± 2.3	3.91 ± 0.1	13.46 ± 0.8	172.3 ± 5.3

Values are means ± SE (n = 6–8). At 10 h the rats were treated with either AT-125 (15 mg kg^{-1}) or saline (NaCl), and at 11, 15 and 19 h they were treated with either GSH (100 mg kg^{-1}) or saline. The rats were killed 24 h after the AT-125 administration.
[a] $P < 0.05$ versus GSH rats.

levels of the GSH- and AT-125-treated rats differed significantly ($P < 0.05$).

Experiment 5: effect of the administration of cysteine

Table 4 shows the effect of the administration of cysteine and GSH on kidney MT and lipid peroxidation. There was no significant effect of the treatments on any of the variables.

Discussion

As expected (Hidalgo *et al.* 1988a, b, 1990a, b), liver MT levels were increased by immobilization stress, an effect clearly higher than that of food and water deprivation alone which *per se* increases liver MT

Table 4. Effect of glutathione and cysteine administration on kidney MT and lipid peroxidation

	MT ($\mu g\,g^{-1}$)	Lipid peroxidation ($nmol\,g^{-1}$)
NaCl	11.0 ± 1.6	269 ± 11.0
Glutathione (100 $mg\,kg^{-1}$)	13.0 ± 1.6	289 ± 8.7
Cysteine 1 (25 $mg\,kg^{-1}$)	11.4 ± 0.8	270 ± 10.2
Cysteine 2 (50 $mg\,kg^{-1}$)	12.0 ± 0.8	284 ± 12.7
Cysteine 2 (acute)	10.4 ± 0.8	267 ± 10.6

Values are means ± SE (n = 6–7). Rats were injected for 4 days with saline (NaCl), GSH (100 $mg\,kg^{-1}$, about 0.325 $mmol\,kg^{-1}$), cysteine 1 (25 $mg\,kg^{-1}$, about 0.206 $mmol\,kg^{-1}$) or cysteine 2 (50 $mg\,kg^{-1}$, about 0.412 $mmol\,kg^{-1}$). Some rats received a single injection of cysteine 2 [cysteine 2 (acute)]. The rats were killed 24 h after the last injection.

levels (Bremner & Davies 1975), indicating a specific effect of stress on liver MT levels. Surprisingly, the opposite effect was seen in kidney MT levels of 18 h stressed rats. This was confirmed in a second, separate experiment (not shown).

To establish whether the observed differences between liver and kidney MT responses to stress might be related to free radical processes, we measured the lipid peroxidation levels, which are known to be consistently increased in the liver, by either stress or starvation (Hidalgo *et al.* 1988a, 1990b). This was confirmed in the present studies, suggesting that stress-induced MT synthesis might be related to lipid peroxidation in the sense that the latter reflects free radical processes. Indeed, basal MT levels are higher in the kidney than in the liver, which might have to do with the higher lipid peroxidation levels of the former tissue. In addition, BSO (see below) increased both kidney lipid peroxidation and MT levels after 2 days of treatment. However, the results do not support such a relation as a general rule. Thus, kidney lipid peroxidation levels were increased by 18h of stress, although to a lower extent than in the liver, whereas kidney MT levels decreased, clearly not supporting a direct relationship between MT and lipid peroxidation in the kidney, as appears to be the case in the liver (Hidalgo *et al.* 1988a, 1990b, Sato & Sasaki 1991).

The present results cannot be explained either by changes in zinc metabolism. Serum zinc levels are decreased by stress, whereas liver cytosolic zinc levels are increased, suggesting the liver uptake of zinc from the serum; given the role of MT in zinc metabolism (Kägi & Kojima 1987), some effect of zinc on liver MT synthesis might be expected. In contrast to the liver, kidney zinc levels were not altered by stress, suggesting that a role of zinc on the observed changes in kidney MT levels during stress is unlikely. We have to keep in mind, however, that the amount of zinc in MT compared with the total cytosolic zinc is small and therefore that changes in MT levels (in these experiments) may not be reflected in total zinc levels.

There is a third factor that can be discussed as being responsible for the effect of stress on MT levels, the adrenocortical hormones glucocorticoids. It is well known that these hormones control the MT gene (Karin *et al.* 1980, Mayo & Palmiter 1981) and that their secretion is increased by stress (Hidalgo *et al.* 1986). Therefore, it is classically assumed that these hormones mediate the effect on MT levels. However, there is some uncertainity about the physiological role of these hormones (see Hidalgo *et al.* 1988b, 1991, 1992 and references therein). In the present report we observe again that ADX increases liver MT levels and that corticosterone reverses that effect. The results for kidney MT levels were in total agreement with those of the liver, suggesting that glucocorticoids would have an inhibitory role on kidney MT regulation in basal conditions. In principle, these hormones should not be responsible for the effect of stress on kidney MT levels, since it is known that they increase kidney MT mRNA levels (Hager & Palmiter 1981, Nebes *et al.* 1988). Although we have no explanation for the differences between liver and kidney MT responses to stress, it is noteworthy to realize that other factors have a similar effect. Thus, it has been described that the tumor necrosis factor (TNF) increases liver MT and decreases kidney MT (Grimble & Bremner 1989). This supports the assumption that MT might be an acute-phase protein (Bremner & Beattie 1990).

It is known that stress decreases liver GSH levels, which might have a effect on MT levels (for further discussion, see Hidalgo *et al.* 1990b). Unfortunately, we could not measure GSH levels in Experiment 1. We did study the putative relationship between MT and GSH in the kidney in other experiments. To this end, we used the administration of BSO because it decreases GSH synthesis (Meister 1988) and that of AT-125 because it decreases GSH degradation (Reed *et al.* 1980). As expected, the administration of BSO decreased kidney GSH levels. In contrast, BSO increased kidney MT levels. These results are consistent with those obtained in the liver (Hidalgo *et al.* 1990b); furthermore, the administration of

cysteine or GSH also increases liver MT levels. Unfortunately, attempts to increase kidney MT levels by the administration of cysteine or GSH were unsuccesful. This suggests that the administered cysteine and the amino acid constituents of GSH (GSH is not effectively transported into most tissues, but is subjected to extracellular breakdown) enter into the liver preferently to the kidney. The fact that liver MT levels were increased by BSO, GSH and cysteine led us to suggest that hepatic MT levels are elevated by increased cysteine pools and that MT, GSH and cysteine are somehow inter-related (Hidalgo *et al*. 1990b). The results obtained for kidney MT with BSO might lead to the same conclusion. Several reports also suggest such a possibility; thus, it has been demonstrated that cysteine-treated rats have higher liver MT levels than control rats (Dudley & Klaassen 1984), whereas Kang *et al*. (1989) and Suzuki & Cherian (1990) have shown that MT levels in cultured human carcinoma cells are increased by BSO in a dose–response manner, and that BSO-treated rats have higher liver and kidney MT levels when injected with cisplatin compared with rats treated with cisplatin alone, respectively. Previously, other authors also considered a relationship between MT and GSH to be feasible in some circumstances (Wong & Klaassen 1981, Kawata & Suzuki 1983).

In this regard, it would be important that the opposite, i.e. decreased cysteine pools, led to decreased MT levels. However, the published literature does not allow us to reach a significant conclusion. First, it is important to realize that there are two sulfhydryl sources for MT synthesis, cysteine and methionine (which is converted to cysteine through the cystathionine pathway) (Stein *et al*. 1987). When the cystathionine pathway is inhibited in rat primary hepatocyte cultures, MT levels induced by zinc tend to decrease if cysteine is the only sulfhydryl source in the culture medium and significantly decrease if it is methionine or if there are no sulfhydryls in the culture medium (Stein *et al*. 1987). In agreement, Gallant & Cherian (1989) showed that the inhibition of the cystathionine pathway decreased liver MT levels in newborn rats. However, Houghton & Cherian (1991) did not find a significant effect in adult rats even at much higher doses. Second, when the dietary intake of sulfhydryls is manipulated, conflicting results are again observed. Gallant & Cherian (1989) and Taniguchi & Cherian (1990) reported that rats fed with sulfhydryl-deficient diets do not modify liver MT levels. In contrast, Sendelbach *et al*. (1990) demonstrated that rats fed with diets deficient in sulfhydryls actually had higher liver MT levels than control rats.

From the above comments, however, it cannot be said that liver and kidney GSH and MT levels are directly linked. Firstly, it is clear that all of the cysteine 'released' in the kidney in the present report by the BSO treatment (about 1.5 μmol g^{-1} kidney) has not gone to MT (which has consumed less than 25 nmol g^{-1} cysteine). The same can be said for the liver (Hidalgo *et al*. 1990b). Secondly, the renal (or hepatic) turnover of GSH and MT are quite different (minutes for GSH, hours for MT). Thirdly, MT and especially GSH are continuously secreted, especially in the liver. In addition, it must be pointed out that it is unclear how increased cysteine levels can increase MT levels, a protein transcriptionally regulated. On the basis of these problems, it will be useful to study the relationship between MT, GSH and cysteine in a more controlled system such as cultured primary hepatocytes.

The present results suggest that there are similarities but also substantial differences between liver and kidney MT regulation. Since the kidney is an important target of cadmium and other metals (Kägi & Kojima 1987), the observed changes in kidney MT levels might have relevance for the toxicity of such metals in some circumstances, i.e. after severe stress or decreased GSH synthesis.

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