0026-895X/89/030398-07$02.00/0

MOLECULAR PHARMACOLOGY, 36:398-404

Quantitative High Resolution ^{1}H NMR Urinalysis Studies on the Biochemical Effects of Cadmium in the Rat

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Received February 10, 1989; Accepted May 15, 1989

SUMMARY

Quantitative changes in the urinary excretion patterns of low molecular weight compounds were followed for up to 30 days after dosing of adult Sprague-Dawley rats with single intraperitoneal injections of $CdCl_2$ (6–24 μmol/kg), using high resolution ^{1}H NMR multicomponent urinalysis. There was a marked reduction in the rate of urinary excretion of citrate, 2-oxoglutarate, and succinate within 4.5 hr of the administration of 24 μmol/kg Cd^{2+}. This continued for up to 4 days after dosing in male rats and was consistent with a renal tubular acidosis, caused by inhibition of carbonic anhydrase. Histological examination of the kidneys showed no evidence of structural abnormalities at any Cd^{2+} dose level. Creatinine excretion was not affected by Cd^{2+} treatment at any dose level but hippurate excretion was significantly reduced. Severe testicular damage was noted within 24 hr of Cd^{2+} treatment at doses of >9 μmol/kg and the degree of damage appeared to be correlated with the presence of large amounts of creatine (up to 20 mM) in the urine. Analysis of homogenates of healthy testicular material indicated the presence of high concentrations of free creatine. Cadmium-induced creatinuria appears to result from direct release of creatine from the necrotic cells of the seminiferous tubules and, hence, the measurement of creatine excretion rates may provide a useful noninvasive indicator of testicular necrosis. Because NMR is nonselective in terms of metabolite detection, this work has shed new light on the changes in urinary composition arising from Cd toxicity. As such, the technique is potentially very valuable in the search for new metabolic markers of toxicity and organ dysfunction.

Administration of cadmium salts to mammals results in rapid accumulation of Cd in the liver, kidney, and pancreas (1, 2). Significant amounts of cadmium may also enter the testis, which is the principal target organ in acute cadmium toxicity (3). After doses of 2 mg of Cd/kg, intraperitoneally, there follows a rapidly progressive hemorrhagic necrosis of the seminiferous tubules, with subsequent testicular atrophy and permanent sterility (4). Following initial exposure by injection, there is usually a period of weeks or months during which cadmium is redistributed in the body, resulting in secondary accumulation of metal in the kidney. This is the critical target organ for cadmium toxicity in low level and chronic oral exposure conditions (5). The renal damage produced by chronic exposure to cadmium is predominantly of a tubular type, in which there is a generalized impairment of solute reabsorption from the glomerular ultrafiltrate. This results in an acquired Fanconi syndrome, in which there is aminoaciduria, phosphaturia, glycosuria, and low molecular weight proteinuria (5). This syndrome has been recorded in human populations experiencing excessive environmental or industrial exposure to cadmium compounds (6), but abnormalities of urinary composition are not normally thought to be associated with acute exposure of laboratory animals to Cd (7).

We have previously shown that NMR urinalysis can provide accurate quantitative data on a variety of low molecular weight compounds present in urine (8, 9). This novel approach appeared to provide a means of detecting acute nephrotoxic lesions produced by experimental mercury poisoning and was at least as sensitive as conventional methods using urinary marker enzymes for assessing nephrotoxicity (10). Perhaps more importantly, by interpretation of the changing patterns of excretion of endogenous urinary metabolites (that were NMR detectable) occurring after exposure, new insights into the molecular mechanisms of toxic action of mercury were obtained (10). Recent studies on a range of nephrotoxins (11, 12) and hepatotoxins (13, 14) have revealed that characteristic patterns of perturbed urinary metabolites, detectable by NMR, can be related to the site, severity, and mechanism of toxicity. In the present study, we have investigated the acute effects of Cd^{2+} treatment on the composition of rat urine, using high resolution proton NMR spectroscopy for urinalysis as well as

This work was supported by grants from the National Kidney Research Fund and Rio Tinto Zinc. We are grateful to the MRC and SERC for use of central NMR facilities. We thank Professor D. R. Wilkie for helpful discussion and Ms. J. Drury and J. Gray for practical assistance.

[1] Unpublished observations.

ABBREVIATION: CA, carbonic anhydrase.

conventional biochemical and histological methods for assessing renal and testicular toxicity. This has yielded new information on the biochemical effects of cadmium on the kidney and testis of experimental rats.

Materials and Methods

Experimental Animals

Adult male or female Sprague-Dawley rats (weight range, 200–350 g) were kept in plastic metabolic cages and provided with food and water *ad libitum* for the duration of the experiments. Urine was collected over ice after filtration through glass wool.

Animal Dosing and Urine Sample Collection

Dose-response effects on urinary metabolism. Fifteen male rats of approximately 250 g (each weighed exactly) were alloted to five treatment groups of three animals each. Rats were acclimatized for 2 days in metabolic cages and were held in a 12-hr light-dark cycle. Each animal was then given a single intraperitoneal injection of $CdCl_2$ (analytical grade; Aldrich Chemicals) in saline solution, equivalent to doses of 0 (control), 0.68, 1.02, 1.35, or 2.70 mg of Cd/kg of body weight (i.e., 0, 6, 9, 12, or 24 μmol of Cd/kg; three animals/group). Urine was collected between 0 and 4.5 hr and from 4.5 to 24 hr after cadmium dosing; the pH and volume of each sample were recorded. Control 24 hr urine samples were collected from each animal before Cd treatment.

After 24 hr, the rats were killed and tissues were removed and prepared for histological examination as follows. Animals were deeply anesthetized with pentobarbital (25 mg intraperitoneally) and the abdominal cavities were opened. The general condition and appearance of the abdominal organs were noted and the kidneys and testes were removed and longitudinally bisected. Tissue samples were then immersed in either Carnoy's or Karnowsky's fixative solution, dehydrated, and embedded in wax or Araldite (15). Small pieces of kidney were also taken and frozen before histochemical preparation to demonstrate the presence/absence of CA, using the method described by Pearse (16). Wax-embedded serial sections (6-μm thick) were stained using hematoxylin and eosin and by the periodic acid-Schiff method. Araldite-embedded material was cut at 1-μm thickness and stained with toluidine blue.

Investigation of sex differences. Control 24-hr urine samples were collected from three male (average weight, 250 g) and three female (average weight, 225 g) Sprague-Dawley rats of the same age and being maintained under identical conditions. The rats were then dosed with 24 μmol of Cd/kg and urine samples were collected 0–24, 24–48, 48–72, and 72–96 hr after dosing. The rats were then allowed to recover for 35 days, when another 24-hr urine sample was collected before reinjection with the same dose of cadmium. Urine samples were then collected 0–24, 24–48, 48–72, and 72–96 hr after the second cadmium injection. At the end of this experiment, the animals were killed and tissues were prepared for histological examination, as described above.

Thirty-day monitoring experiment after single exposure to Cd. The longer term effects of a single dose of $CdCl_2$ were investigated using three male and three female rats (approximate average weights, 300 and 250 g, respectively), each given 24 μmol of Cd/kg. After collection of an initial control 24-hr urine sample before cadmium dosing, the rats were injected and urine samples were collected 0–24, 24–48, 48–72, and 72–96 hr after dosing, and then further 24-hr collections were made at 6, 7, 8, 9, 10, 14, 15, 16, 23, 24, and 30 days after dosing. After 30 days, the animals in the first group were killed and samples of testis and kidney were taken for histology. The second group was reinjected with 24 μmol of Cd/kg at day 30 after the first dose and urine samples were collected for an additional 48 hr (i.e., two 24-hr collections).

^{1}H NMR Spectroscopy of Urine and Testis Samples

Spectra were recorded on Bruker WH400 and AM500 spectrometers operating at 400 and 500 MHz, respectively, at 25°, using 0.4 ml of urine diluted with 0.1 ml of ^2H_2O (lock signal) containing sodium-*d*-(trimethylsilyl)propionate to give a final concentration of 2 mM, hence providing a quantitation standard and an internal chemical shift reference (δ = 0 ppm). For each sample, 64 free induction decays were collected into 16,384 computer points (acquisition time, 1.7 sec), using 50° pulses and a total pulse recycle time of 5.6 sec to allow full T_1 relaxation. A continuous secondary irradiation field was applied at the resonance frequency of water in order to suppress the intense signal.

Control rat testes were removed from freshly killed animals and were immediately homogenized with an equal volume of ^2H_2O in the presence of 1 mM 1-fluoro-2,4-dinitrobenzene (Sanger's reagent, a potent inhibitor of creatine kinase activity). Hahn spin-echo spectra of the testicular homogenates were measured at 500 MHz using a 2τ relaxation delay of 120 msec to attenuate broad resonances from macromolecules via T_2 relaxation and so give a spectrum containing signals from the more abundant low molecular weight metabolites (17).

^{31}P NMR Spectroscopy of Rat Testis

Spectra of whole intact testes and testicular homogenates were recorded on a Bruker WM200 spectrometer with a dedicated 80.95 MHz probe (15-mm internal diameter) using broad band proton decoupling, a 50° pulse width, and a delay between pulses of 5 sec to allow T_1 relaxation.

Results

Histopathological and Post-Mortem Observations

General observations. The general condition of the abdominal organs of Cd-treated rats was examined at post-mortem. The liver, kidneys, and heart of each rat appeared normal in all treated rats, irrespective of dose. At Cd doses of 12 or 24 μmol of Cd/kg, about 2–5 ml of clear, nonpurulent, serous exudate was found in the abdominal cavities of rats 24 hr after injection; the intestines of these animals appeared pale and peristaltic movements were weak and irregular at post-mortem. Animals killed more than 3 days after administration of 9, 12, or 24 μmol of Cd/kg had dense fibrous adhesions around the gut, which also encapsulated the stomach and spleen, and connected the lobes of the liver. The extent and density of these lesions was higher in animals receiving the highest doses of Cd. There was no histological indication of kidney damage at any Cd dose level.

Testicular lesions. Testicular damage was also apparent at necropsy, the severity of which increased with Cd dose and varied with time after dosing. No histopathological abnormalities were observed in the testes of rats dosed with 0 or 6 μmol of Cd/kg. In rats treated with 9 μmol of Cd/kg, occasional necrotic cells were seen, with evidence of hydropic degeneration in the spermatogonia of a small proportion (<10%) of the seminiferous tubules; the gross appearance of the testis on hemisection was normal. Twenty-four hours after doses of 12 or 24 μmol of Cd/kg, the testes appeared swollen and dark red, due to severe hemorrhagic necrosis, and the tissue was very friable. At these higher doses the seminiferous tubules lacked turgor and failed to bulge outwards from the cut surface of the tunica albugenea, as was seen in healthy control animals.

Detailed histological examination of the testicular material revealed severe degeneration and necrosis of the germinal cell line of the seminiferous tubules 24-hr after the administration of 12 or 24μmol of Cd/kg. The regular succession of differentiating cells in the stratified epithelium that was observed in control rat testis was disrupted and spermatozoa were absent. Sertoli cells appeared to be largely unaffected but the remaining spermatogonia were vacuolated and had pyknotic nuclei. Mural

thrombi were visible in the larger blood vessels and there was marked extravasation of blood into the peritubular connective tissue. Aggregates of cellular debris were visible in the lumens of most seminiferous tubules. Three days after administration of 12 or 24 μmol of Cd/kg, vascular thrombosis was more marked and few germinal cells were observed, autolytic changes being almost complete. Necrosis and autolytic changes in the seminiferous tubules appeared to be maximal between 48 and 72 hr after Cd treatment. Seven days after injection of 24 μmol of Cd/kg, the testes were much smaller than those of control animals, and the contents of the tunica albugenea were much harder and more compact due to extensive postnecrosis calcification, which reached a maximum 30 days after dosing (confirmed by von Kossa staining for calcium). Thirty five days after 12 or 24 μmol of Cd/kg treatment, no intact germinal cells were visible, the tubular contents being replaced by a dense matrix of calcified and fibrinous debris. A very strong Von Kossa reaction for calcium was obtained in these sections. Peritubular fibrosis was also evident, with intense fibroblast activity and collagen deposition. Our histopathological observations were consistent with those of earlier workers (4).

^{1}H NMR of Rat Urine: Cd Dose-Response Effects

A total of 42 control 24-hr urine samples were collected from male Sprague-Dawley rats and metabolites were measured by ^{1}H NMR spectroscopy. The concentrations of 21 low molecular weight metabolites were determined in each sample by comparing the areas of proton resonances with those of the standard added sodium d_4-(trimethylsilyl)propionate methyl signal and are shown in Table 1. ^{1}H NMR spectra of urine samples taken from control rats and healthy humans have been described previously (8, 10, 12). The "aliphatic" region of a typical 400 MHz spectrum of control rat urine is shown in Fig. 1A. Treatment of rats with cadmium chloride results in major qualitative and quantitative changes in this region in the NMR spectra of urine samples taken from these animals, as illustrated in Fig. 1, B–E. Significant changes in the intensities of resonances from these metabolites were observed after doses of 9 μmol of Cd/kg and above. The most striking changes include the disappearance of signals from the tricarboxylic acid cycle intermediates citrate, succinate, and 2-oxoglutarate after doses of 9 μmol of Cd/kg and above and the appearance of intense resonances from creatine at doses of 12 μmol of Cd/kg and above. Hippurate resonances (e.g., the doublet at δ 7.88), normally relatively intense in control rat urine samples (10), were also reduced in intensity in urine samples from Cd^{2+}-treated animals (spectra not shown). Urinary excretion rates were calculated from NMR concentration data and expressed as μmol of each metabolite/kg of body weight/24 hr. The dose-response effects of Cd on 24-hr urinary excretion of these metabolites by male rats are shown in Fig. 2. In rats given 12 μmol of Cd/kg, the excretion of citrate, succinate, 2-oxoglutarate, and hippurate was reduced to approximately half that of controls (Fig. 2). Creatinine excretion rates did not vary significantly with Cd dosing and hippurate levels were only depressed at the highest Cd dose (Fig. 2). At a dose of 24 μmol of Cd/kg, major changes in the excretion rates of these compounds were detectable as early as 4.5 hr after dosing, although creatinine excretion rates were constant, suggesting little effect of Cd on renal blood flow or glomerular filtration rates (Fig. 3).

TABLE 1

Total mean urinary excretion (mean ± 1 SE) of 21 metabolites by control adult male Sprague-Dawley rats (n = 42), determined by proton NMR measurements

Metabolite	Measured NMR signal[a]		Excretion	
			Mean	Range
		ppm	μmol/24 hr	
Acetamide	CH_3 (s)	2.04	7.0 ± 2.2	1.0–8.6
Acetate	CH_3 (s)	1.93	13.4 ± 3.6	0.8–33.2
Acetoacetate	CH_3 (s)	2.34	2.2 ± 0.2	0.1–8.6
Alanine	CH_3 (d)	1.48	1.0 ± 0.9	0.2–1.9
Allantoin	CH (s)	5.39	64.0 ± 6.0	16.3–178.4
Trimethylamine *N*-oxide[b]	CH_3 (s)	3.27	9.8 ± 2.4	2.7–20.5
Citrate	$[CH_2]_2$ (AB)	2.64	68.8 ± 6.8	19.2–178.2
Creatine	CH_2 (s)	3.95	ND[c]	ND
Creatinine	CH_2 (s)	4.07	52.3 ± 3.1	41.6–73.4
Dimethylglycine	$(CH_3)_2$ (s)	2.89	5.8 ± 1.8	1.3–10.0
Dimethylamine	CH_3 (s)	2.73	11.6 ± 2.5	4.8–22.1
Formate	CH (s)	8.47	3.6 ± 1.5	0.6–21.6
Glucose[d]	α-CH (d)	5.24	4.4 ± 0.8	1.1–8.3
Glycine	CH_2 (s)	3.57	1.2 ± 0.5	0.3–2.6
Hippurate	$[CH]_2$ (d)	7.88	23.4 ± 2.5	9.6–60.8
3-D-hydroxybutyrate	CH_3 (d)	1.25	0.9 ± 0.6	0.2–1.5
2-Oxoglutarate	CH_2 (t)	2.47	28.2 ± 3.8	9.6–64.8
Lactate	CH_3 (d)	1.34	5.1 ± 2.2	0.8–20.4
Methylmalonate	CH_3 (d)	1.26	7.6 ± 3.7	0.1–60.1
Succinate	$[CH_2]_2$ (s)	2.42	7.4 ± 1.6	2.2–12.2
Taurine	CH_2 (t)	3.45	10.2 ± 2.9	1.6–19.2

[a] Identity of resonance used in quantitative studies and chemical shift at pH 7.
[b] This peak overlapped with that of betaine, which may give a small contribution to the measured intensity.
[c] ND, not detectable in NMR spectra.
[d] Based on resonance from α-anomeric form (normal equilibrium proportion 30%).

Acute Cd Toxicity: Sex Differences in Urinary Metabolite Profiles

After injection of 24 μmol of Cd/kg, proton NMR urinalysis measurements showed that both male and female rats exhibited a significant decrease in the excretion of citrate, 2-oxoglutarate, succinate, and hippurate (Figs. 4–7, respectively). In female rats, minimum excretion rates for Krebs's cycle intermediates were observed during the 0–24-hr period, whereas male rats showed minima 24–48 hr after dosing. There was a gradual increase in the urinary excretion rates of these compounds toward control levels up to 96 hr after dosing in female rats, whereas the excretion rates in males remained suppressed (Figs. 4–6). Creatinine excretion rates were not significantly altered throughout this period (Fig. 7). Hippurate excretion reached a minimum between 24 and 48 hr after dosing but recovered toward control values during the 72–96-hr collection (Fig. 7). Creatine excretion reached a maximum 48–72 hr after Cd injection in male rats, reaching about 0.5 mmol/kg/hr, and then declined during the 72–96 hr collection (Fig. 8). There was very little creatinuria in male rats 144–168 hr after Cd dosing (Fig. 8). Female rats excreted <5% as much total creatine as males over the 0–96-hr period following Cd treatment (Fig. 8). After day 6 (144–148 hr) and from days 7 to 30, all rats, male and female, had normal NMR spectral profiles of urine (e.g., Fig. 1E) and all metabolites were excreted at the same rate as controls (data not shown). NMR spectra of urine did not show any indications of aminoaciduria or glycosuria typical of proximal tubular damage as found after comparable doses of mercury (II) chloride (10, 12). In male rats redosed with Cd^{2+} 30 days after the initial 24 μmol/kg injection, there was virtually no creatinuria detectable (Fig. 8), but depression of the excretion of Kreb's cycle intermediates was the same as that seen with the initial Cd dose (data not shown).

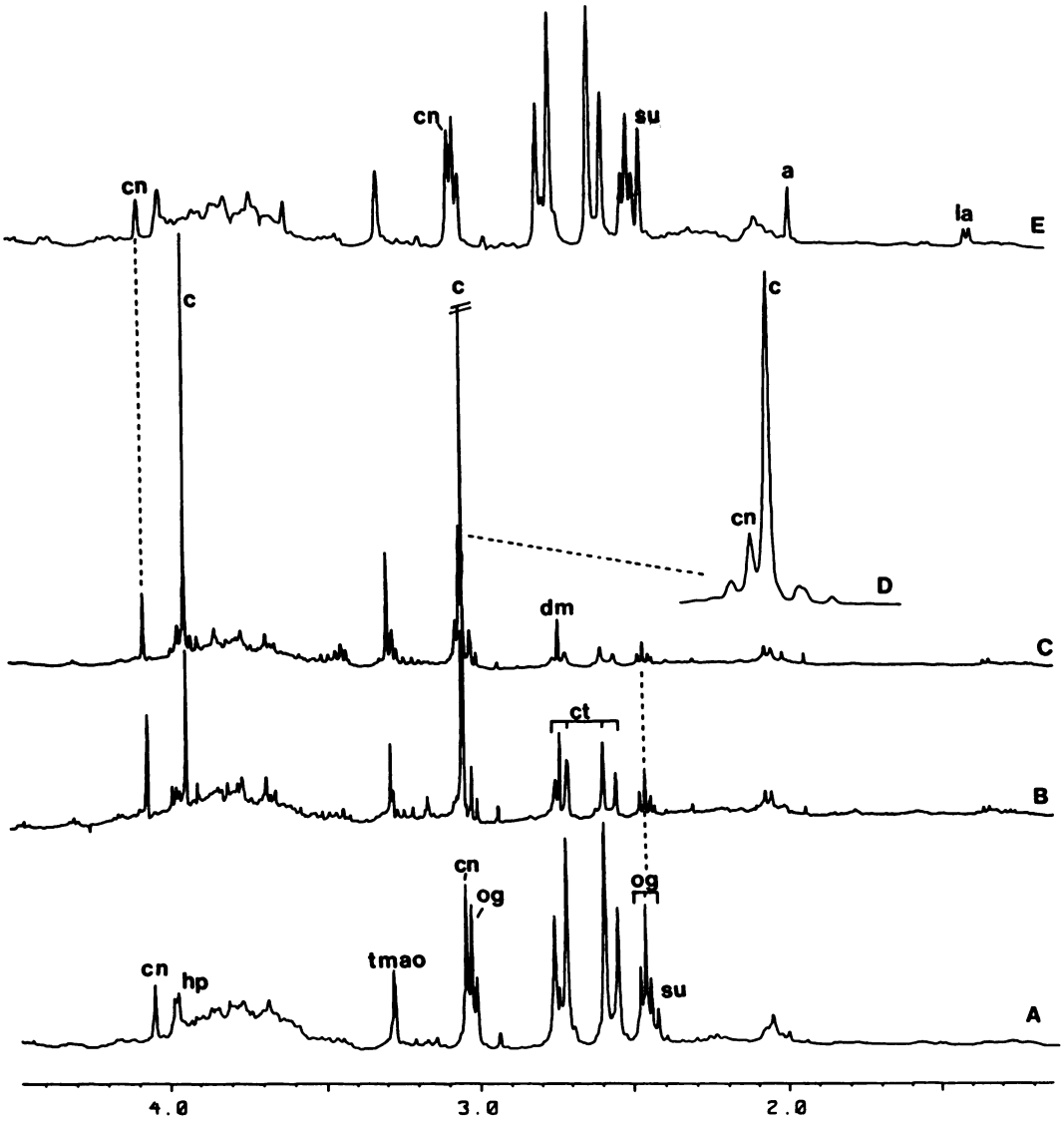

Fig. 1. Typical partial 400 MHz spectra (δ 0.5–4.5 ppm) or urine collected from a male rat for 24 hr before dosing (control) with 24 μmol of Cd/kg of body weight (A), for 0–24 hr after dosing with Cd (B), for 24–48 hr after dosing with Cd (C and D) (D is an expansion of the chemical shift region between 2.98 and 3.1 ppm on half the vertical scale, showing the relative intensities of creatinine and creatine), or for 6 to 7 days (24-hr collection) after dosing with Cd (E). Assignment of resonances: *su*, succinate; *og*, 2-oxoglutarate, *cn*, creatinine (two signals from the CH_3 and CH_2 protons at δ 3.07 and 4.07 ppm, respectively); *c*, creatine (two signals from the CH_3 and CH_2 protons at 3.04 and 3.95 ppm, respectively); *tmao*, trimethylamine-*N*-oxide; *ct*, citrate; *dm*, dimethylamine; *a*, acetate; *la*, lactate; *hp*, hippurate.

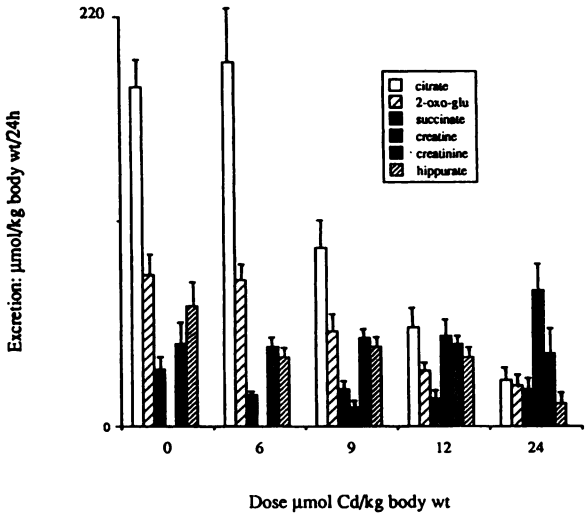

Fig. 2. Dose-response effects of Cd on the excretion rates of various urinary metabolites by male rats. *Bars* show mean + 1 SE for three rats for each dose. *2-oxo-glu*, 2-oxoglutarate.

Discussion

We have previously shown that ^{1}H NMR spectroscopy of urine can be used effectively for the detection and assessment of mercury (II) chloride-induced acute renal damage and that the changing patterns of metabolites excreted in the urine reflect subcellular toxic events in the renal tubules (10–12). A similar approach has been used here to investigate the biochemical effects of cadmium exposure in rats. Cadmium, like mercury, is a group IIb metal and has chronic nephrotoxic potential as well as its toxic effects on the testis in mammals (5). It is, therefore, worthwhile to compare the effects on the urinary composition from rats exposed to each metal at similar dose levels. Both metals result in major changes in the excretion of urinary metabolites that can be detected by ^{1}H NMR. However, the patterns of induced change are dissimilar, indicating that at the molecular level the cellular effects of these metals are very different (Table 2). With cadmium urinalysis indicates that both renal and nonrenal effects can occur, whereas with mercury the biochemical changes mainly reflect acute changes in renal tubular cell metabolism and function (10). In single doses, mercury is much more toxic to the kidney (2) and the consequent acute proximal tubular necrosis is well documented

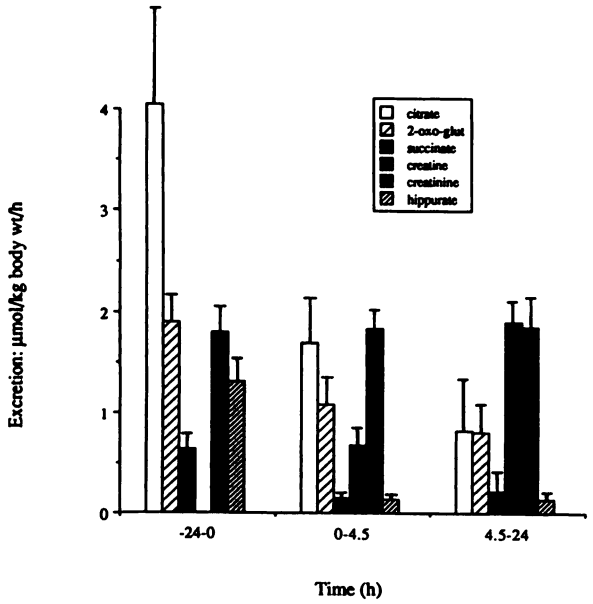

Fig. 3. Early time-course effect of 24 µmol of Cd/kg of body weight on the urinary excretion of various metabolites by male rats. *Bars* show mean + 1 SE for three rats. Average hourly excretion values have been calculated for the two collection periods to make the data readily comparable. *2-oxo-glut*, 2-oxoglutarate.

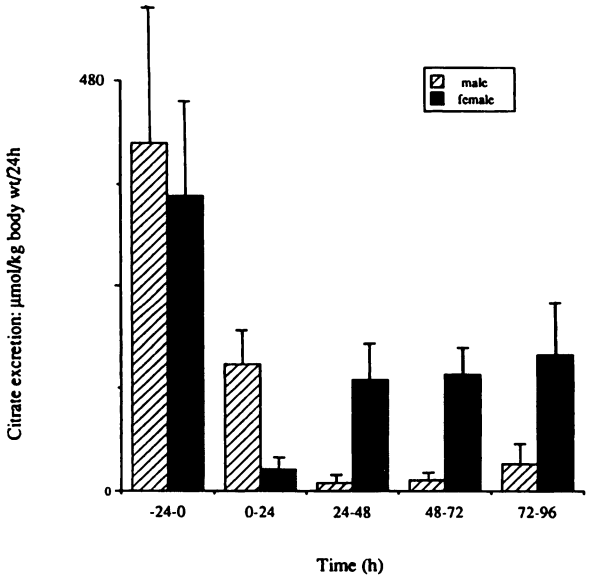

Fig. 4. Time-course effect of 24 µmol of Cd/kg of body weight on the urinary excretion of citrate by male and female rats. *Bars* show mean + 1 SE of the mean for three rats.

(3). After single doses of cadmium, few nephrotoxic effects have been reported and the composition of urine from Cd-treated animals has been reported to be normal (7). The chronic nephrotoxicity of cadmium salts and their acute testicular toxicity are well known (5). The changes in urinary composition after a single cadmium dose that have been revealed by ^{1}H NMR spectroscopy in this study are, therefore, entirely new.

A striking effect of Cd^{2+} treatment is a sudden and major reduction in the excretion of the tricarboxylic acid cycle intermediates citrate, 2-oxoglutarate, and succinate, which are all normally abundant in rat urine (12). This effect persisted for several days in both male and female rats, the effect being more

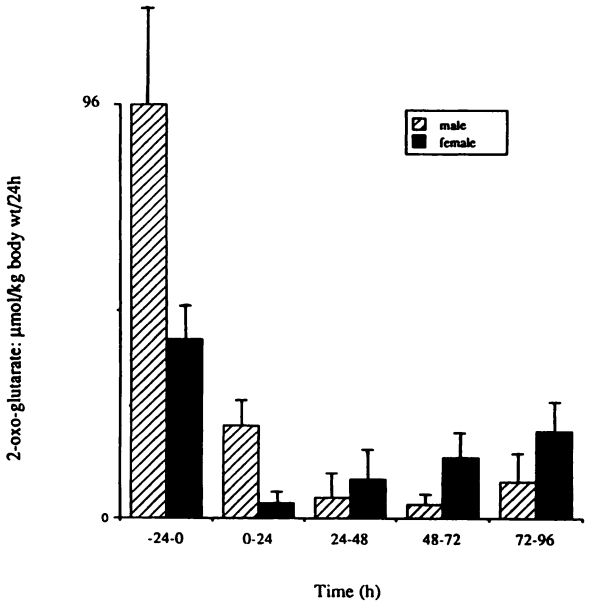

Fig. 5. Time-course effect of 24 µmol of Cd/kg of body weight on the urinary excretion of 2-oxoglutarate by male and female rats. *Bars* show mean + 1 SE for three rats.

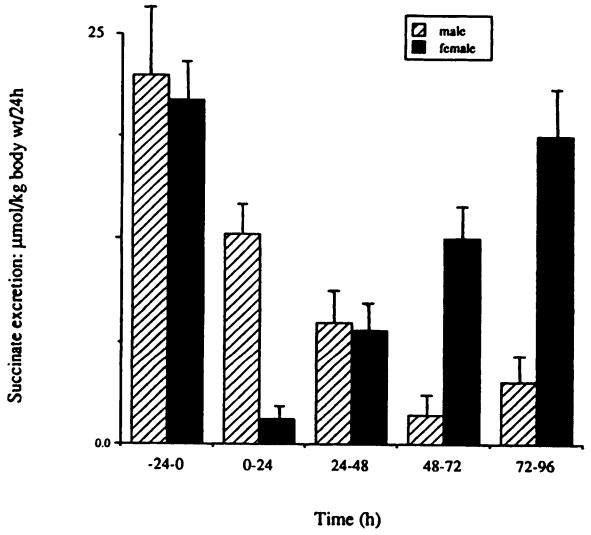

Fig. 6. Time-course effect of 24 µmol of Cd/kg of body weight on the urinary excretion of succinate by male and female rats. *Bars* show mean + 1 SE for three rats.

pronounced and persistent in males. The rate of intracellular citrate metabolism in the kidney plays a major role in determining the amount of citrate excreted in the urine (18). Metabolic acidosis causes cytoplasmic pH and bicarbonate to increase and, with this, an increase in the mitochondrial pH gradient. This change stimulates the tricarboxylate carrier, speeding entry of citrate into the mitochondrial matrix compartment and, hence, reducing the level of cytoplasmic citrate. Peritubular citrate uptake is then reduced and the citrate clearance rate is reduced (18). Other organic acids utilized in the Kreb's cycle (of which succinate and 2-oxoglutarate are abundant in body fluids) are metabolized in the same way as citrate under varying acid-base balance conditions (19). We attribute the reduction in the excretion of citrate, 2-oxoglutarate, and succinate after cadmium exposure to a marked inhi-

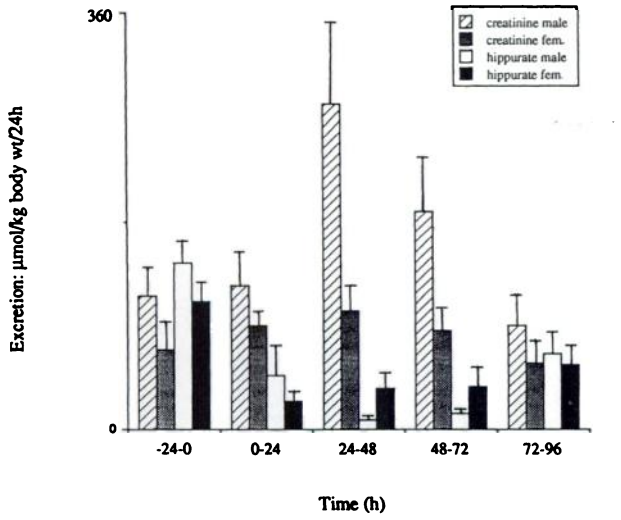

Fig. 7. Time-course effect of 24 µmol of Cd/kg of body weight on the urinary excretion of hippurate and creatinine by male and female rats. *Bars* show mean + 1 SE for three rats.

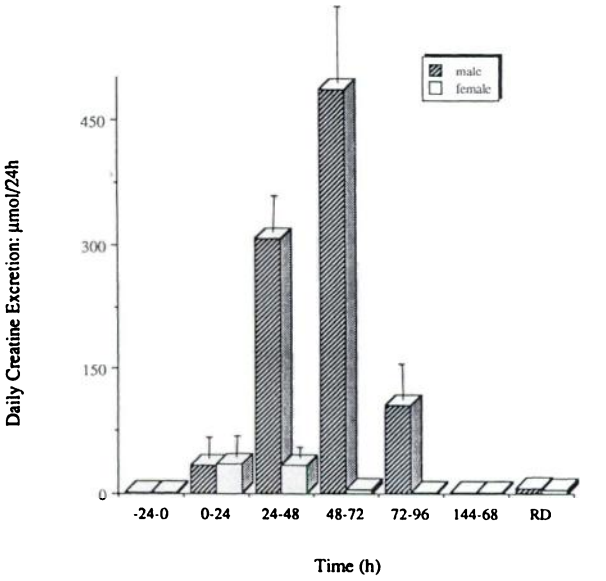

Fig. 8. Time-course effect of 24 µmol of Cd/kg of body weight on the 24-hourly urinary excretion of creatine by male and female rats. *RD*, rats redosed with 24 µmol of Cd/kg of body weight 30 days after initial dosing with Cd (24-hr collection period). *Bars* show mean + SE for three rats.

bition of renal CA that results in renal tubular acidosis. Using a standard histochemical method (17), no CA activity could be detected in rats 24 hr after treatment with 24 µmol Cd/kg of body weight; strong CA staining was observed from proximal tubular profiles of control animals (data not shown). CA controls the hydration of carbon dioxide and the dehydration of carbonic acid and, hence, bicarbonate reabsorption from the renal tubular lumen. Under conditions of CA inhibition, renal tubular acidosis occurs, thus affecting the flux of metabolites such as citrate and 2-oxoglutarate across the mitochondrial membrane and through the tricarboxylic acid cycle itself (18). The action of potent CA inhibitors such as acetazolamide can be shown to markedly reduce the urinary excretion of these organic acids, although, unlike with cadmium, the effects only persist for a maximum of 48 hr (11). In the case of mercury

TABLE 2

Comparison of the effects of single equimolar intraperitoneal doses (24 µmol of metal/kg of body weight) of $CdCl_2$ and $HgCl_2$ on the 0–24-h urinary excretion of endogenous metabolites by male Sprague-Dawley rats

+, Significantly increased relative to controls ($p < 0.05$); −, significantly reduced relative to controls ($p < 0.05$); NS, no significant difference between controls and treated.

Metabolite	Hg^{2+}*	Cd^{2+}
Acetate	+	NS
Alanine	+	NS
Citrate	−	−
Creatine	NS	+
Creatinine	−	NS
Formate	+	NS
Glucose	+	NS
Glycine	+	NS
Hippurate	NS	−
Lactate	+	NS
2-Oxoglutarate	+	−
Succinate	+	−

* See Ref. 10 for details.

exposure in rats, we detected inhibition of CA by ^{1}H NMR spectroscopy of urine (10), but superimposed upon CA-related biochemical effects were those of a mitochondrial lesion, specifically the inhibition of malate and succinic dehydrogenases by mercury. This resulted in a reduction of urinary citrate clearance, consistent with renal tubular acidosis, but also elevated urinary 2-oxoglutarate and succinate levels, which are normally associated with alkalosis (10). These studies show that there is considerable utility in the application of proton NMR urinalysis to study indirectly, but noninvasively, renal tubular mitochondrial function, CA activity, and general renal substrate metabolism.

The other striking feature of the ^{1}H NMR spectra of urine from Cd-treated male rats was the presence of intense signals from creatine, which is frequently not detectable in control animals. Creatinuria was dose related (Fig. 2) and appeared to be maximal between 24 and 48 hr after dosing, corresponding very well with the occurrence and maximal severity of cadmium-induced testicular damage. Female rats excreted much less creatine after Cd treatment. We decided to investigate further the possibility that urinary creatine levels were directly related to testicular damage and so provide a possible noninvasive indicator of acute testicular damage. Previous studies have shown that the testis is very rich in endogenous creatine, with levels being as high as those found in skeletal or heart muscle (20). Muscle creatine is found mainly as the phosphorylated derivative phosphocreatine, which acts as a fast-acting energy reserve when it is cleaved by creatine kinase. This enzyme is of very low activity in the testis. The earlier report (20) did not distinguish between testicular creatine and phosphocreatine. Our studies using ^{1}H NMR spectroscopy of testicular homogenates show that the creatine exists in the testis as the free base at concentrations of 10–20 mM (data not shown), also no ^{31}P phosphocreatine signals were detected in rat testis or homogenates (data not shown). The function of free creatine in the testis is unknown. Other workers using noninvasive surface-coil ^{31}P NMR measurements of rat and human testis (21) have shown that very little phosphocreatine exists in the healthy tissue and, hence, this precludes creatine functioning as a high energy phosphate store as it does in skeletal muscle. Preliminary experiments on the effects of dietary restriction

on creatinuria[1] indicate that mild creatinuria in both male and female rats may be due to increased muscle turnover. It also appears that Cd dosing results in a temporary reduction in food intake, which could explain the transient creatinuria observed here in female rats. Surgically orchidectomized rats showed little creatinuria after Cd dosing, but ligation of the testicular arterial plexus resulted in massive creatinuria, strongly suggesting that the male pattern creatinuria was related to the testicular toxicity.[1]

The important findings of this study include the indirect detection by ^{1}H NMR urinalysis of biochemical effects of cadmium on renal CA, in the absence of structural damage to the kidney. Furthermore, it appears that the massive creatinuria found in male rats after Cd treatment is strongly related to the degree of testicular damage determined histopathologically. As such, urinary creatine measurements may be useful in the detection of testicular toxicity caused by other xenobiotics under toxicological screening tests. This work illustrates the potential that NMR offers for the rapid screening and assessment of acute testicular toxicity in experimental animals and NMR may find widespread application for testing novel therapeutic agents.

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