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Renal amino acid transport in immature and adult rats during chromate and cisplatinum-induced nephrotoxicity

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Summary. The effects of sodium dichromate (chromate; 1 mg/100 g b. wt. s.c.) and cisdiamminedichloroplatinum(II) (CP; 0.6 mg/100 g b. wt. i.p.) on renal amino acid excretion and plasma amino acid composition were investigated in 10- and 55-day-old anaesthetised rats. On the basis of diuresis experiments on conscious rats the mentioned doses and times (1st day after chromate in both age groups and in 10-day-old rats after CP and 3rd day after CP in adult rats) were found out to be optimal for the characterisation of amino acid transport after heavy metal poisoning. Interestingly, in conscious 10-day-old rats chromate nephrotoxicity is not detectable after 1 mg/100 g b. wt. whereas all of the other experimental groups showed nephrotoxic effects of chromate and CP in conscious rats. Urine volumes are lower, but not significantly, in anaesthetised immature rats, independently of the administered nephrotoxin. But GFR is significantly lower in 10-day-old rats, both in controls and after CP, whereas after chromate GFR is significantly reduced only in adult rats and age differences disappeared. In principle the renal fractional excretion (FE) of amino acids was distinctly higher in immature rats as a sign of lower amino acid reabsorption capacity. Nevertheless, the amino acid plasma concentrations were relatively high in immature rats. However, both chromate and CP did not distinctly influence amino acid plasma concentrations. But in both age groups the administration of chromate and CP significantly decreased amino acid reabsorption capacity (increase in FE) as a sign of nephrotoxicity, most pronounced in adult rats after CP. The investigation of renal amino acid handling confirms (1) that both CP and chromate are nephrotoxins, (2) that CP was more nephrotoxic in 55-day-old animals compared to immature rats as could be demonstrated before using other parameters for nephrotoxicity testing and showed (3) that determination of renal amino acid handling is a highly sensitive marker for nephrotoxicity testing, especially in immature rats.

Keywords: Amino acids – Cisplatinum – Chromate – Nephrotoxicity – Amino acid transport – Kidney – Ontogeny – Rats

Abbreviations: CP – cisplatinum; FE – fractional excretion; γ-GT – γ-glutamyltransferase; GFR – glomerular filtration rate; β-NAG – N-acetyl-beta-D-glucosaminidase

Amino acids: Ala – alanine; Arg – arginine; Asn – asparagine; Asp – aspartic acid, Gln – glutamine; Glu – glutamic acid; Gly – glycine; His – histidine; Ile – isoleucine; Leu – leucine; Lys – lysine; Met – methionine; Phe – phenylalanine; Ser – serine; ß-Ala – ß-alanine; Tau – taurine; Thr – threonine; Tyr – tyrosine; Val – valine

Introduction

Little is known about the influence of heavy metals on the renal handling of amino acids and about renal amino acid excretion as a marker for acute nephrotoxicity. The tubular reabsorption of amino acids occurs first of all at the luminal membranes of the proximal renal tubules (Silbernagl, 1992). This region is directly in contact with the glomerular ultrafiltrate and, therefore, heavy metals could damage amino acid transporters specifically. Foulkes (1987) reported that heavy metal intoxication not only inhibits transport of amino acids at the brush border, but also prolongs their transepithelial transit time and increases the size of cellular transport pool for a given reabsorbed load. Administration of heavy metals induces a condition resembling the Fanconi syndrome. An apparently uncompetitive inhibition of reabsorption of amino acids was observed 2 days or longer after injection into rabbits. Metals cause relatively specific effects, manifested by lesions at the brush border membrane. Functional lesions after exposure to heavy metals are reviewed in relation to renal amino acid transport. Such effects may vary from metal to metal but they do not appear to represent general cytotoxicity (Foulkes, 1983). The glomerular sensitivity to mercury may indicate an important target region of the nephron in the development of nephrotoxicity, but e.g. mercury affects mainly the proximal tubule (Wilks et al., 1990). Furthermore, after cadmium administration, marked increases in urinary amino acid excretion were observed (Min et al., 1987), also uranium causes significantly higher excretion of some amino acids (Thun et al., 1985), iron chelate led to a loss of renal proteins (Uchida et al., 1995), urinary excretion of amino acids increased after exposure of rats to inhalation of nickel carbonyl up to 7-fold (Horak and Sunderman, 1980), cisplatinum therapy in humans reduced glomerular filtration rate and caused a decrease in renal amino acid reabsorption (Rossi et al., 1994; Arndt et al., 1999) and sodium chromate pretreatment inflicted necrotic damage mainly in the proximal part of the proximal convoluted tubules in rats (Sparrow et al., 1988; Appenroth et al., 1991), the region of most effective amino acid reabsorption.

Amino acid handling within the kidney develops after birth and reaches adult values at one month of age (Fleck, 1992). Tiruppathi and co-workers (1987) confirmed that the activities of the Na^+ gradient-driven amino acid transport systems in renal brush border membrane vesicles were higher in the adult than in the suckling rats. The accumulation of the beta-amino acid

taurine is higher in adult rat renal brush border membrane vesicles than in nursing animals (Chesney et al., 1987). Based on studies on very young animals, the inadequate activity of some enzymes and the immaturity of the transport processes seem to be responsible for age differences in amino acid reabsorption capacity (Schreier, 1986). The renal reabsorption of amino acids in canine pups was incomplete and after 3 up to 8 weeks adult pattern of reabsorption was present (Bovee et al., 1984). There are some exceptions from the rule: e.g. the transport of glycine matures later in development (Grosvenor and Zeman, 1983). Possibly amino acid reabsorption capacity is correlated with glomerular filtration: GFR develops after birth up to day 30. Thereafter, GFR remains relatively constant for up to 3 months and drops continuously until the 8th month of age (Fleck, 1999).

Therefore, age differences in the effect of heavy metals on renal amino acid transport as shown for thallium (Tl) have to be considered for other metals, too. As shown previously (Fleck and Appenroth, 1996), the nephrotoxic effects of Tl are more pronounced in adult rats compared to immature animals. The smaller morphologic destructions in the medulla and Henle's loop of 10-day-old rats after Tl compared to adult animals may be caused by lower Tl concentrations in renal tissue reflecting the immature tubular secretion capacity in young rats (Appenroth et al., 1995).

For further charactersation of age differences in heavy metal nephrotoxicity we investigated the influence of a single administration of chromate (Bradberry and Vale, 1999) and cisplatinum (Li et al., 1994) known to act nephrotocxic on the renal handling of amino acids in immature (10-day-old) and adult (55-day-old) rats to clarify (1) further age differences in nephrotoxicity, (2) to characterise similarities and differences between the effects of various heavy metals on renal amino acid transport, and (3) to find out whether or not the determination of renal amino acid handling is a suitable marker for nephrotoxicity as described by Fent et al. (1988).

Material and methods

Animals and treatment

Investigations were performed on Wistar rats (Han: Wist) of our institute's own outbreed stock. At the beginning of the experiments animals were 10- and 55-day-old and the average body weight was 21 ± 2 and 172 ± 8 g, respectively. Adult female rats and young rats of both sexes were used. The litters were reduced to 6 offsprings per mother. Rats were kept in plastic cages under identical conditions (12/12 h light/dark cycle), in environmentally controlled rooms (22 ± 2°C temperature, 50% ± 10% humidity), including free access to standard diet Altromin 1316R (Altromin GmbH & Co. KG, Lage, Germany) and tap water. The experiments were permitted in accordance with the German law in regard to humane treatment of research animals.

Test substances

Sodium dichromate (chromate): $Na_2Cr_2O_7 \times 2H_2O$ (Serva, Heidelberg, Germany); 1 mg/100 g b.wt. was dissolved in 1 ml saline/100 g body wt. and administered subcutaneously (s.c.).

Cis-diamminedichloroplatinum(II) (cisplatinum, CP; Jenapharm GmbH, Jena, Germany) 0.6 mg/100 g b. wt. were dissolved in 5 ml saline/100 g b. wt. and administered intraperitoneally (i.p.).

Control: saline 1.0 (for chromate) s.c. or 5.0 ml/100 g b. wt. (for CP) i.p.

Experimental design

The test substances were injected once at the 10th or 55th day of life.

Experiments on conscious rats: Nephrotoxicity was characterised over a period of 5 days in diuresis experiments after a single administration of chromate or CP. Control rats received the same volume of the corresponding vehicle. Urine samples were collected at different times after the administration of chromate or CP. Urination was induced by slight manual pressure on the suprapubic region at the beginning and at the end of the 1 h collecting period. To obtain sufficient amount of urine in 10-day-old rats, urine samples of 2–3 animals were pooled for one determination. Volume and total protein concentration were measured.

Experiments on anaesthetised rats: Diuresis experiments were performed at the day of maximal nephrotoxic effect as found in experiments on conscious rats and on the basis of previous experiments. It could be shown that at days 1 and 3 (cp. Fig. 1) maximal nephrotoxic effects occur in immature and in adult rats, respectively (Appenroth et al., 1988; Appenroth et al., 1994). The rats were anaesthetised with ketamine (Ursotamin® Serumwerk Bernburg, Germany, 7.5 mg/100 g b.wt.) and xylazine (Ursonarkon® Serumwerk Bernburg, Germany, 1.2 mg/100 g b. wt.). Both substances were administered intramuscularly. A catheter was placed in a tail vein (adult animals) or in the left jugular vein (immature rats). The animals were then infused isotonic saline containing 4 g/l fluorescein isothiocyanate (FITC)-inulin (Bioflor, Uppsala, Sweden) at a priming rate of 3 ml/100 g b. wt. per 1 hour for 15 minutes and then at 2 ml/100 g b. wt. per 1 hour for the remainder of the experiment. Thereafter a polyethylene catheter was inserted into the urinary bladder. To minimise urine collecting periods for the determination of GFR and fractional amino acid excretion (FE), urine was collected in three 20-minute periods (55-day-old rats) or in an one-hour period (10-day-old rats). In previous experiments it could be shown that under these experimental conditions both hematocrit (Fleck et al., 1992) and blood pressure (Fleck and Bräunlich, 1986) remain nearly constant during the clearance study. In the middle of each period and at the end of the experiment blood was collected from the retrobulbar plexus.

Analytical methods

Total urinary protein was measured by the Coomassie blue dye-binding method (Bradford, 1976).

Glomerular filtration rate (GFR) was determined by inulin clearance. Inulin concentration was measured spectrofluorimetrically using FITC-inulin (Sohtell et al., 1983) in blood and urine samples. Fluorescence was measured at 480 nm excitation and 520 nm emission wave length in a HITACHI F-2000 spectrofluorimeter.

Amino acid determination: The determination of amino acids by column chromatography with fluorescence detection is based on that developed by Roth and Hampai (1973) and has been described in detail elsewhere (Silbernagl, 1983). Proteins were removed from urine and plasma samples by administration of trichloroacetic acid. After centrifugation, the supernatant was neutralised by adding 0.4 N NaOH. Then the samples were diluted with citrate buffer and analysed by HPLC on an amino acid analyser (Knauer, Berlin, Germany) with o-phthalaldehyde as a fluorescent amino ligand (Roth, 1971). Calibration runs were performed with freshly prepared amino acid solutions composed of analytical grade amino acids (Serva, Heidelberg, Germany).

Mode of presentation and statistics

In the figures amino acids are arranged in the following order: acidic (Asp, Glu), basic (Arg, Lys, His), and neutral aliphatic (Gly, Ala, Ser, Thr, Val, Leu, Ile, Met) neutral aromatic (Phe, Tyr) and neutral amides (Asn, Gln) as well ß-alanine (ß-Ala) and taurine (Tau), described to be transported by the same carrier different from the others (Silbernagl, 1992), are given additionally. Results are summarised as means + S.E.M.; n = 6 in 55-day-old rats and n = 3–4(6) in 10-day-old animals. Differences between the experimental groups were analysed by Student's t-test and considered statistically significant when $p \leq 0.05$ (FE, GFR, urine volume, body weight) or $p \leq 0.001$ (amino acid plasma concentration) considering the well known high interindividual variability of this parameter (Lingard et al., 1974).

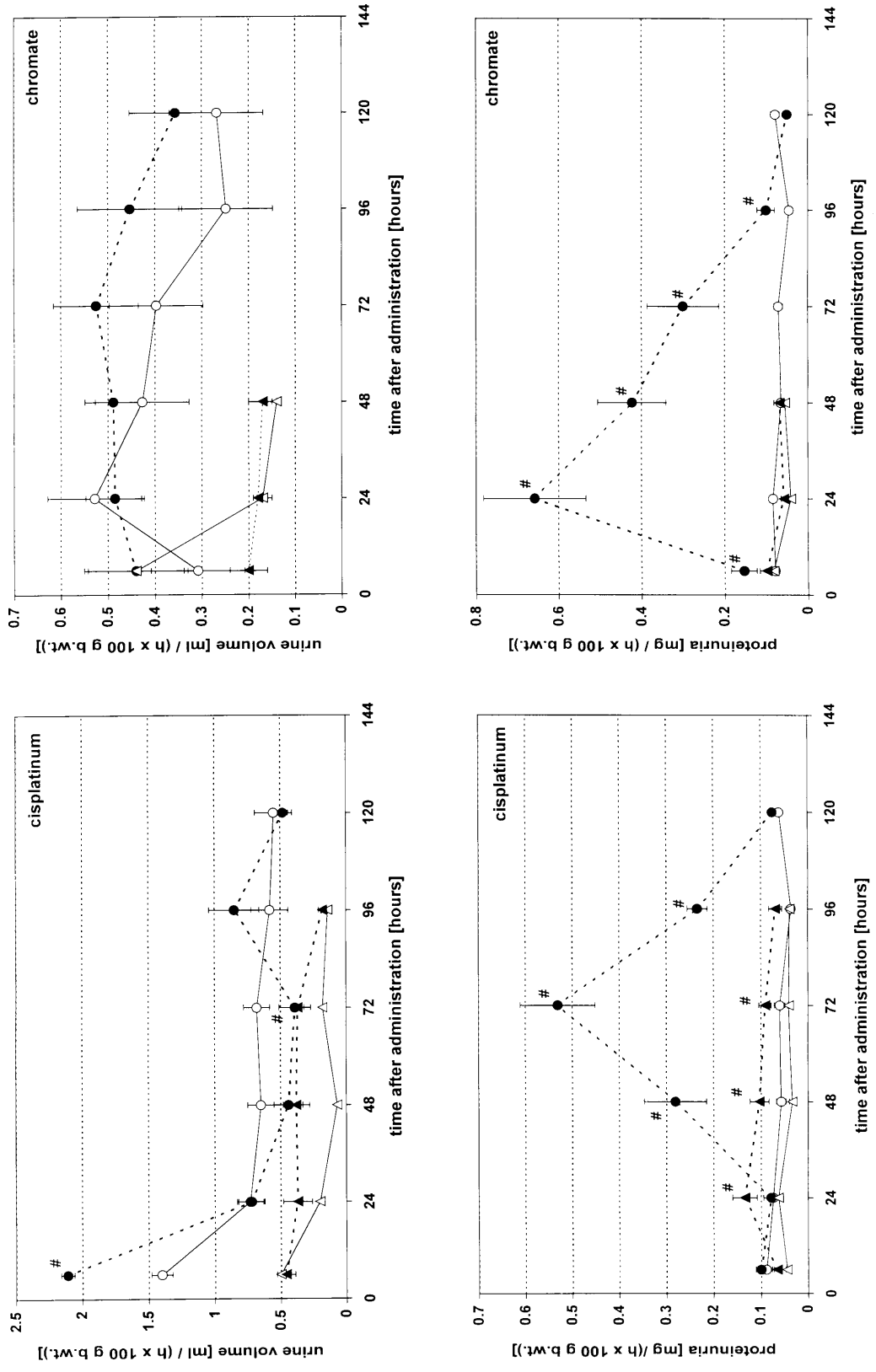
Results

The injection of well characterised doses of chromate or CP into conscious 10- and 55-day-old rats confirmed that CP is more nephrotoxic in adult rats (reduction in body weight gain, poly- and oliguria, proteinuria at day 2–4, maximal at day 3 after the administration of CP and between 6 hours and 4 days, maximal after 24 hours after chromate) than in immature animals (Fig. 1). In the young age group the chromate dose tested (1 mg/100 g b. wt.) was in conscious rats without signs of nephrotoxicity, despite in previous experiments after higher doses (2 mg/100 g b. wt.) under these experimental conditions chromate nephrotoxicity could be found in immature animals, too (Appenroth et al., 1991).

In further experiments on anaesthetised rats the renal handling of endogenous amino acids was investigated under the influence of the two heavy metals. For this purpose in Fig. 2 the urine flow rates and GFR values as basic parameters are shown: Urine volumes are lower, but not significantly, in anaesthetised immature rats, independently of the administration of nephrotoxins. However, the GFR is significantly lower in 10-day-old rats, both in controls and after CP, whereas after chromate GFR is significantly reduced only in adult rats and age differences disappeared. On the other hand, CP has no influence on GFR, neither in adults, nor in immature animals.

In Table 1, the control values of amino acid plasma concentrations and renal fractional amino acid excretions (FE) are given. The well known age differences could be stated: the tubular reabsorption capacity of young rats is immature and, therefore, FE values are higher compared to adult animals for most of the endogenous amino acids. Surprisingly, in these experiments the amino acid plasma levels of immature rats were relatively high in comparison to adults and to previous findings (Fleck, 1992). Nevertheless, because in all experiments age-matched controls have been used, these differences do not influence the interpretation of our results.

The most important results of this study are shown in figures 3 and 4: both after administration of chromate (Fig. 3) and CP (Fig. 4) the fractional excretion of endogenous amino acids is significantly enhanced as a result of the nephrotoxicity of these two substances. After pretreatment with CP this effect occurred in all cases and was more pronounced in adult animals (mean


chromate
urine volume [ml / (h x 100 g b.wt.)]
time after administration [hours]
proteinuria [mg / (h x 100 g b.wt.)]
cisplatinum
urine volume [ml / (h x 100 g b.wt.)]
proteinuria [mg /(h x 100 g b.wt.)]
0
24
48
72
96
120
144
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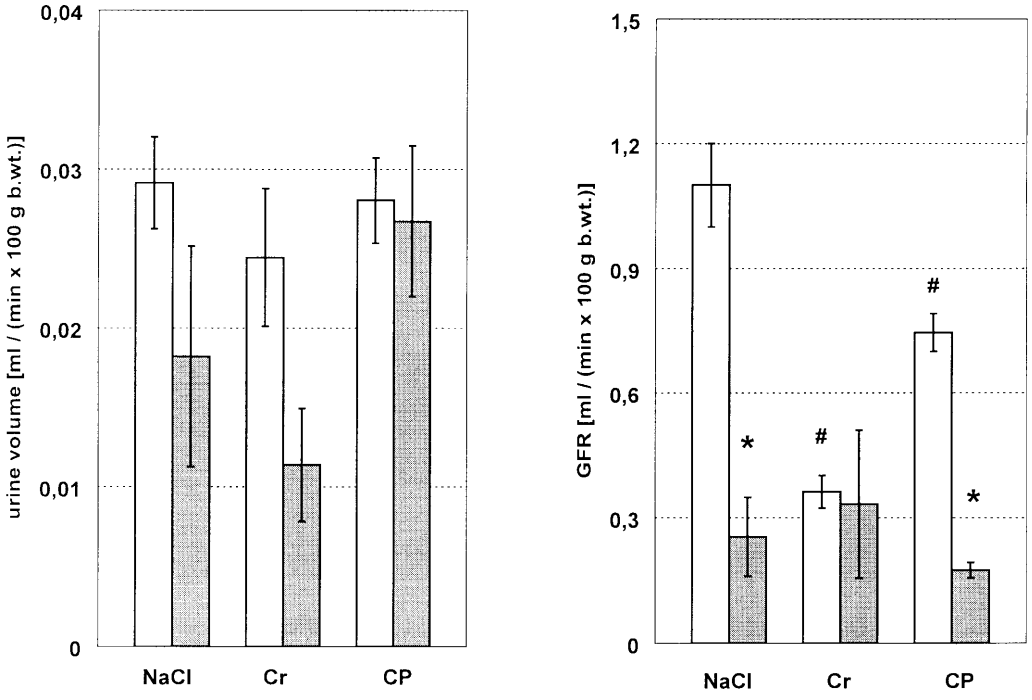

Fig. 2. Influence of 0.6 mg cisplatinum (*CP*) or 1 mg chromate (*Cr*) per 100 g b. wt. on urine flow and GFR in 10- (black columns) and 55-day-old (open columns) anaesthetised rats 24 hours after the administration of the nephrotoxic agent (CP in 55-day-old rats: 72 hours). *NaCl* = controls; arithmetic means ± S.E.M., n = 12 (55 days) or 3–6 (10 days). * – significant age differences ($p \leq 0.05$); # – significant influence of chromate or cisplatinum ($p \leq 0.05$)

increase of FE-values about 5.1-fold) compared to 10-day-old rats (mean increase only about 2.5-fold). But also after single administration of chromate the FE-values in 14 of 19 amino acids (10-day-old rats) and in 15 of 19 amino acids (55-day-old rats) were enhanced, more pronounced in 10-day-old rats. In each age group this increase was significant in five cases. These findings are in good accordance with the reduction of GFR after chromate in adult rats. Compared to the lack of effect of chromate in 10-day-old rats in the experiments on conscious rats (Fig. 1), the amino acid reabsorption seems to be influenced by chromate in immature animals at lower doses than total protein excretion.

Discussion

It was the goal of this study to answer three questions: (1) Is the measurement of the renal handling of amino acids a suitable marker for the determination

Fig. 1. Influence of 0.6 mg cisplatinum or 1 mg chromate per 100 g b. wt. on urine volume and renal protein excretion in 10- (▲; △) and 55-day-old conscious rats (●; ○) at different times after administration (open symbols = controls). Arithmetic means ± S.E.M., n = 6. # – significant influence of chromate or cisplatinum ($p \leq 0.05$)

Table 1. Age differences in amino acid plasma concentrations and fractional amino acid excretions in control rats treated with NaCl. Arithmetic means ± S.E.M.; n = 12 (55-day-old rats) or 3–4 (10-day-old rats)

Amino acid	Plasma concentration [μM]		Fractional excretion [%]	
Age	10-days	55-days	10-days	55-days
Acidic				
Asp	98 ± 12	53 ± 2	3.39 ± 0.26	3.43 ± 0.45
Glu	248 ± 33	123 ± 4 *	2.45 ± 0.70	3.71 ± 0.63
Basic				
Arg	313 ± 53	180 ± 9	2.96 ± 1.85	0.94 ± 0.09
Lys	401 ± 39	310 ± 16	1.04 ± 0.15	0.81 ± 0.08
His	401 ± 33	201 ± 7*	2.74 ± 0.52	1.31 ± 0.14*
Neutral				
Gly	380 ± 24	172 ± 4*	6.71 ± 2.71	2.75 ± 0.37
Ala	499 ± 66	274 ± 13	1.91 ± 0.46	1.37 ± 0.15
Ser	435 ± 66	204 ± 10	2.03 ± 0.37	1.38 ± 0.21
Thr	578 ± 64	288 ± 9*	2.27 ± 0.53	1.44 ± 0.17
Val	473 ± 122	224 ± 6	8.49 ± 2.26	0.96 ± 0.13*
Leu	194 ± 27	186 ± 12	1.38 ± 0.19	0.45 ± 0.06*
Ile	99 ± 14	115 ± 8	1.13 ± 0.24	0.56 ± 0.10
Met	89 ± 8	46 ± 2*	2.50 ± 0.25	2.01 ± 0.23
Phe	536 ± 13	150 ± 11*	8.42 ± 2.31	4.21 ± 0.62
Tyr	416 ± 44	85 ± 4*	2.24 ± 0.25	1.32 ± 0.14*
Asn	80 ± 10	43 ± 1*	3.13 ± 0.29	1.72 ± 0.19*
Gln	982 ± 109	421 ± 28	2.13 ± 0.49	0.97 ± 0.13
Others				
ß-Ala	38 ± 7	12 ± 1	7.35 ± 2.02	6.55 ± 0.77
Tau	441 ± 41	127 ± 14*	12.71 ± 4.71	9.44 ± 1.68

* = significant differences between the two age groups (plasma concentrations: $p \leq 0.001$; FE: $p \leq 0.05$).

of acute nephrotoxicity? (2) Do chromate and cisplatinum have toxic effects on renal tubular amino acid reabsorption? And (3) are these effects age dependent?

Ad 1.) Is the measurement of the renal handling of amino acids a suitable marker for the determination of acute nephrotoxicity?

The known nephrotoxicity of CP and chromate, detected with other methods in the own laboratory (cp. Appenroth et al., 1988, 1994) and by other authors, e.g. Li et al. (1994) and Bradberry and Vale (1999), respectively, is reflected also by reduction in renal reabsorption of amino acids. This decrease in amino acid reabsorption capacity can be seen earlier (with respect to time after administration and to dose) than changes in other functional renal parameters like changes in urine flow rate or proteinuria. Therefore it can be stated that the investigation of amino acid transport in the kidney can be used for nephrotoxicity screening, too, with the restriction that the measurement of amino

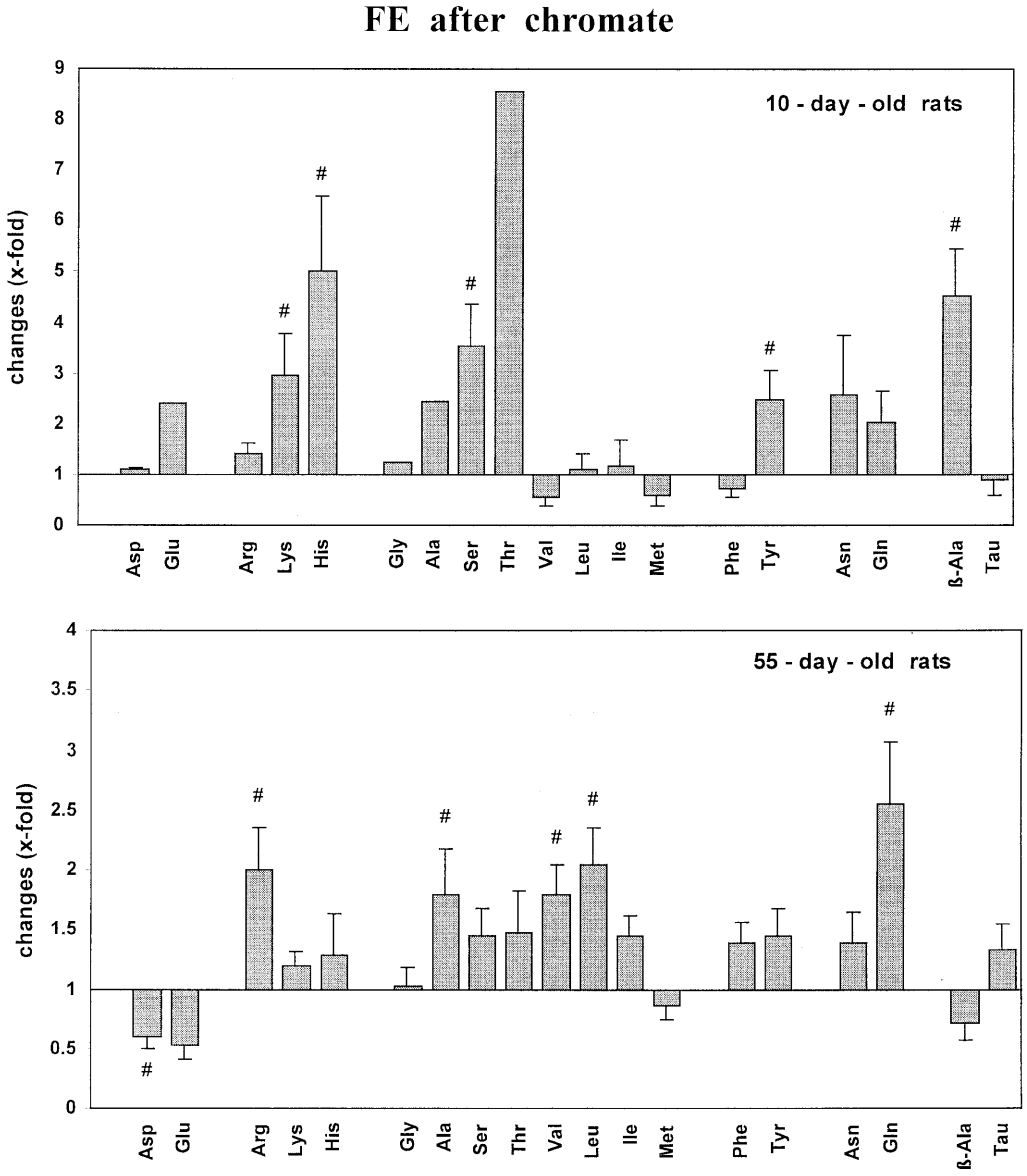

Fig. 3. Influence of 1 mg chromate per 100 g b. wt. on renal fractional excretion (*FE*) of endogenous amino acids in 10- and 55-day-old anaesthetised rats 24 hours after administration. Arithmetic means ± S.E.M., n = 6 (55 days) or 3–4 (10 days). # – significant influence of chromate ($p \leq 0.05$)

acid clearance is relatively time consuming and expansive. Nevertheless, this is in good accordance with previous results obtained using thallium as nephrotoxin (Fleck and Appenroth, 1996): despite under normal circumstances more than 98% of filtered amino acid load is reabsorbed in the proximal tubules (Silbernagl, 1992) the increase in FE after administration of heavy metals is significant. The functional reserve of amino acid reabsorption is extremely high. Therefore it is very difficult to prove changes in amino acid

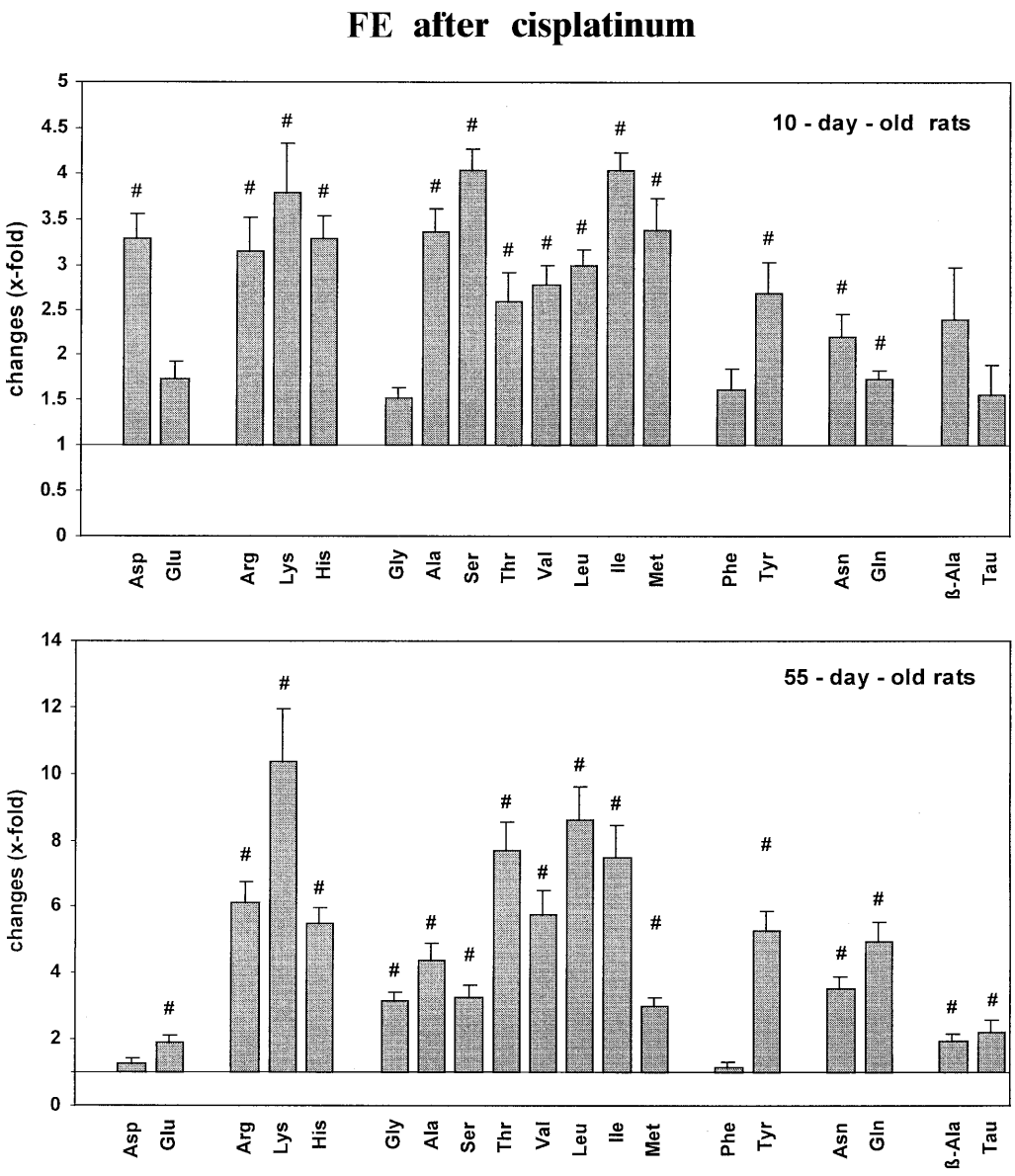

Fig. 4. Influence of 0.6 mg cisplatinum per 100 g b. wt. on renal fractional excretion (*FE*) of endogenous amino acids in 10- and 55-day-old anaesthetised rats 24 and 72 hours after administration, respectively. Arithmetic means ± S.E.M., n = 6 (55 days) or 3–4 (10 days). # – significant influence of cisplatinum ($p \leq 0.05$)

reabsorption capacity (FE) in vivo without exogenous amino acid administration (Fleck, 1992), whereas it is possible after amino acid loading (Fleck et al., 1997). Concluding from our results the determination of renal amino acid handling could be helpful in the characterisation of nephrotoxicity. This investigation could specifically reveal toxic injuries at the luminal membrane of the tubuli at which amino acid reabsorption is located (Palacin et al., 1998). This is a further evidence that the sensitivity of functional parameters is prior to that of morphological investigations (cp. Appenroth et al., 1995).

Ad 2.) Do chromate and cisplatinum have toxic effects on renal tubular amino acid reabsorption?

The fractional excretion of amino acids is the ratio between renal amino acid clearance and GFR. The calculated value considers changes in amino acid plasma concentration and is a stable parameter for tubular amino acid transport. The fractional excretion of amino acids is enhanced after both chromate and cisplatinum, especially in adult animals. In contrast to thallium nephrotoxicity (Fleck and Appenroth, 1996), the reabsorption of nearly all amino acids is disturbed after the two heavy metals. Concerning CP nephrotoxicity in both adults and immature individuals these findings confirm literature data (cp. Jongejan et al., 1986), but with respect to chromate, especially in 10-day-old rats, our amino acid data are completely new.

On the other hand, the administration of chromate and CP has nearly no influence on the plasma concentrations of amino acids. Therefore, the alterations of urinary excretion of amino acids was apparently mediated by nephrotoxicity rather than by mobilisation of amino acids from tissues, since plasma concentrations of amino acids were not significantly affected by exposure to the two heavy metals. These results support the conclusion that a probenecid-sensitive transport process, described by De Ceaurriz and Ban (1991) is involved in the renal toxicity of chromate. The results obtained by Bomhard et al. (1990) indicated a characteristic dose- and time-dependent pattern of chromate nephrotoxicity with primarily glomerular damage, but proximal tubular parts were also affected, as could be demonstrated by histopathological changes (Appenroth et al., 1990). Cellular proliferation and fibrosis were observed 4–5 days after chromate (Sparrow et al., 1988). The excretion of β-2-microglobulin was compared to the excretion of the enzyme β-NAG and amino acids in rats treated with chromate more pronounced and amino acid excretion showed little sensitivity against chromate (Viau et al., 1986).

As mentioned in the introduction, concerning toxic effects of heavy metals on renal tubular amino acid transport little is known in the literature (Berndt and Ansari, 1990). Kim et al. (1990) studied effects of cadmium intoxication on renal transport systems for different amino acids and found that cadmium impairs various Na^+-amino acid co-transport systems in the renal brush border membrane, which leads to panaminoaciduria. Furthermore, renal epithelial amino acid concentrations are found to be changed in mercury-induced acute renal failure (Duran et al., 1990). After intravenous administration, mercury rapidly depressed amino acid transport but no effect of cadmium was seen for at least 6 h (Foulkes, 1991).

Ad 3.) Are these effects age dependent?

Beside age dependent differences in the effects of nephrotoxins on the kidney, age dependent differences in amino acid transport capacity has to be considered. For example, aromatic L-amino acid decarboxylase develops with age, though some activity can already be detected at birth (Soares da Silva et al., 1995). Also dipeptide-proton co-transport activity in rat renal brush border membrane vesicles is developing, starting at 7 days after birth. In addition,

Tiruppathi et al. (1987) have confirmed that the activities of the Na^+ gradient-driven glucose and amino acid transport systems in renal brush border membrane vesicles were higher in the adult than in the suckling rats. Furthermore, it was found that renal fractional amino acid excretion rose significantly with increasing age in humans (Nadvornikova et al., 1991) and in rats (Fleck, 1992). This phenomenon is due to a decrease in their effective tubular reabsorption. A significant correlation was found between FE_{Na} and FE of most amino acids. These findings support the assumption that changes in tubular Na^+ transport probably participate in the changes of tubular amino acid transport in elderly individuals.

It could be demonstrated that nephrotoxicity of both chromate and CP is more severe in adult than in young rats as could be shown previously for thallium (Fleck and Appenroth, 1996). That means, mature kidney function seems to be necessary for complete heavy metal kidney damage. Jongejan et al. (1986) described the effect of cisplatinum on the glomerular filtration rate and effective renal plasma flow of 3-week-old and adult rats. The authors assumed that this is due to the comparatively larger renal mass in relation to body weight in the young animals. Furthermore, the difference in nephrotoxicity between young and adult rats was due to the renal handling of cisplatinum. There was a marked difference in renal platinum concentration: in young rats renal platinum concentration was only about one half of that in adult rats (Appenroth et al., 1988). On the other hand, platinum excretion was higher in immature animals and, therefore, its reabsorption was lower in this age group. Nevertheless, age different changes in glomerular filtration rate and urine volume, in renal excretion of β-NAG (Matsuoka et al., 1986) or γ-GT (Minakami, 1992) as well morphological changes like lysosomal enzyme release (Kobayashi et al., 1989; Appenroth et al., 1990) after administration of CP has to be considered as explanation for age differences in nephrotoxicity. Altogether, age differences in nephrotoxicity of heavy metals are not unique and depend on the metal, on the accumulation of the respective metal in the kidney and on age dependent changes in amino acid transport capacity. Therefore, the effects of heavy metals on the kidney have to be determined for each metal separately and global prediction of nephrotoxicity is not possible.

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