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Consequences of renal mass reduction on amino acid and biogenic amine levels in nephrectomized mice

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Summary. Amino acid and biogenic amine changes were investigated in nephrectomized mice ten days postsurgery. Uremic mice exhibited changes in amino acid concentrations in plasma, urine and brain. Particularly plasma methionine, citrulline and arginine levels were significantly enhanced in nephrectomized mice compared to controls whereas serine was decreased. Urinary excretion of methionine, citrulline and alanine was higher in nephrectomized mice compared to controls whereas many amino acids were increased in brain of nephrectomized mice. Brain and urinary amino acid changes were more pronounced in the 75% than in the 50% nephrectomized mice. Brain norepinephrine and dopamine and its metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid were significantly increased whereas serotonin was decreased comparing the 75% nephrectomized mice to the sham-operated mice. This study demonstrates that at very early stages of renal insufficiency, specific amino acid and biogenic amine changes occur in plasma, urine and brain. These alterations might depend qualitatively and quantitatively on the degree of functional renal mass reduction.

Keywords: Amino acids – Biogenic amines – Renal insufficiency – Mice

Introduction

The kidney plays a leading role in AA metabolism (Cohen and Kamm, 1976). It contains large numbers of enzymes that mediate the synthesis or degradation of AAs (Remesar et al., 1983). AAs are actively transported into renal tubule cells and most of them are reabsorbed along the entire proximal tubule (Bergeron and Morel, 1969; Mitch and Chesney, 1983). Alterations in plasma and urine AA levels were observed in children and in elderly patients with renal failure (RF) (Broyer et al., 1980; Ceballos et al., 1990; Carr et al., 1994) as well as in uremic rats and dogs (Kopple and Sukemoto, 1980; Kikuchi

et al., 1984; Levillain et al., 1997). Usually these abnormalities seen in patients with RF are related to inadequate nutrient intake, impaired renal excretion and acquired metabolic disturbances (Kopple et al., 1978; Yu et al., 1994). Essential AAs (EAAs), cannot be synthesized at a rate adequate to meet metabolic requirements therefore they must be derived from diet. Non-essential AAs (NEAAs) and other AAs, can be synthesized in vivo by amination of carbohydrate and fat residues. EAAs might therefore behave differently from NEAAs in RF (McGale et al., 1972).

RF also has an effect on AA levels and related compounds in brain (Kikuchi et al., 1983; De Deyn and Macdonald, 1990), which may contribute to the development of neurological symptomatology (Ksiazek, 1979) and/or pathogenesis of uremic encephalopathy (Biasioli et al., 1984; 1986; De Deyn et al., 1992). Some AAs act as precursors of brain amines thus the pathogenic mechanism underlying uremic encephalopathy may include alterations in the biogenic amine (BA) system (Gessa and Tagliamonte, 1974).

The AA and cerebral biogenic amine (BA) equilibrium seem to depend, at least partly, on normal functioning of the kidney. The purpose of this study is to examine AA (EAAs, NEAAs and others) profiles in relation to different degrees of RF. As such data are presently lacking, we set up this study using a mouse model for RF that was developed in our laboratory (Al Banchaabouchi et al., 1998). This paper presents the first study of AA changes in uremic mice.

Materials and methods

Animals and surgery

Young adult male mice (Swiss-Webster × C57BL hybrids) weighing around 20–26 g were used. Three experimental groups were considered: the control group (A) was sham-operated and two groups (B, C) were nephrectomized (NX) by a technique described earlier (Al Banchaabouchi et al., 1998). Mice were anesthetized with Hypnorm®/midazolam (1 part Hypnorm®, 1 part Dormicum® and 2 parts water for injection, dose 4 ml/kg i.p) (Bertens et al., 1993). The sham operation consisted of a small bilateral dorsal flank incision to expose the kidneys. In group B, the right kidney was removed after ligature of the renal artery, the renal vein and the ureter (~50% NX). In group C the right kidney was removed as in group B but in addition the contralateral kidney was partially infarcted by ligating the anterior renal artery branch (~75% NX). Animals were housed under normal conditions with a 12 hour light/dark cycle and free access to water and food (rat and mouse chow, containing 16.5% protein). During the first 8 days postsurgery the mice were kept in standard cages. On day 9, mice were placed in metabolic cages (Tecnilab, 3542 AB Utrecht, Netherlands) for urine collection over a 48-hour period. Mice were compared 10 days postsurgery.

Collection and preparation of plasma and urine samples

From each experimental animal, urine was collected in a metabolic cage adapted for mice. On the 11th day postsurgery, mice were anesthetized and subsequently killed by exsanguination via a heparinized needle placed in the abdominal aorta. Samples of arterial blood were collected into heparinized vacutainer tubes. Samples were centrifuged at 700 × g at 4°C for 10 min. Five μl of plasma was used for urea determination. The remaining plasma was used for creatinine (CTN) and AA analysis.

Preparation of brain samples

Brains were removed and stored at −75°C till neurochemical analysis. Frozen brains were divided into two equal halves by sectioning along the sagittal midline. At the time of analysis, for measuring AA concentration, the left hemisphere was homogenized in 1 ml HPLC water at 0°C with a "tissue tearor" (model 985, Biospec Products, Bartlesville, USA). The probe was washed immediately with 1 ml of a 300 g/l trichloroacetic acid solution at 0°C and added to the homogenate which resulted in a protein precipitation after Vortex-mixing. After centrifugation (100,000 × g for 30 min. at 4°C) the clear supernatant was used for AA analysis.

Concentrations of the BAs and metabolites were determined in brain homogenates of the right hemisphere. Frozen brain tissue was weighed and homogenized with the tissue tearor in 3.5 ml ice-cold 0.1 M HCl containing 0.1% $Na_2S_2O_5$ (w/v) and 0.01% Na_2EDTA (w/v) (antioxidant mixture). The stator was washed with 3.5 ml 0.1 M HCl containing 0.1% $Na_2S_2O_5$ (w/v) and 0.01% Na_2EDTA (w/v). The wash fluid was added to the homogenate. The homogenate was centrifuged (4°C, 21,000 g, 30 min) to remove the precipitated proteins and cell debris. The supernatant was diluted 4 times with 0.1 M HCl containing 0.1% $Na_2S_2O_5$ (w/v) and 0.01% Na_2EDTA. This dilution was filtered through a Millipore 0.22-μm filter (Ultrafree®-MC, Millipore, Bedford, MA, USA). Finally, 5 μl of the resulting filtrate was injected into the analytical system. The mobile phase was a 95% sodium-phosphate-citrate buffer (100 mM phosphoric acid, 100 mM citric acid, 20 mg/l Na_2EDTA, 400 mg/l octanesulphonic acid, pH 3) and 5% methanol.

Determination of CTN and urea

For CTN analysis, plasma and urine were deproteinized by mixing equal volumes of a 200 g/l trichloroacetic acid solution with plasma or urine. Samples were centrifuged (Beckman microfuge E, Beckman Instruments, Inc, Palo Alto, California 94304, USA) at 15,850 × g ad 4°C. The supernatant was used for CTN determination using a Biotronic LC 5001 (Biotronik, Maintal, Germany) amino acid analyser adapted for guanidino compound determination as described in detail earlier (Marescau et al., 1992). Urea nitrogen was determined with diacetylmonoxime as described by Ceriotti (1971).

Amino acid analysis

For determination of AAs in plasma and urine, samples were deproteinized using a sulfosalicylic acid solution: 200 μl urine or plasma was vortexed with 50 μl of 10% sulfosalicylic acid solution. Samples were centrifuged (Beckman microfuge E, Beckman Instruments, Inc, Palo Alto, California 94304, USA) at 15,850 × g and at 4°C. Clear supernatant was diluted with sample buffer (0.12 N lithium citrate buffer, pH 2.20).

Amino acids were separated over a cation exchange column using lithium citrate buffer. For colorimetric detection the ninhydrin method was used (Biotronik LC 6001, amino acid analyser, Biotronik, Maintal, Germany). Chromatographic conditions and characteristics have been described in detail by Pei (1994).

Biogenic amines analysis

For determination of the BAs and their catabolites the method described by Cheng et al. (1993) has been used. The compounds have been separated with a BAS 200_B chromatograph (Bioanalytical Systems, West Lafayette, IN, USA). For electrochemical detection a dual glassy carbon working electrode was used. The standard equipment was adapted using a microbore reversed-phase column (Hypersil C18 BDS, 3 μm, 150 mm × 1.0 I.D.) from LC Packings (Zurich, Switzerland). A splitter system was used (Acurate™,

LC Packings, Zurich, Switzerland). The flow rate of the pump was 0.4 ml/min. The splitter system allowed a flow of 40 μl/min passing through the separation column.

Standard products were purchased from RBI (Research Biochemicals International, Natick, MA, USA). Dihydroxybenzylamine and 5-hydroxy-N-methyltryptamine were used as internal standards. All the other chemicals and solvents were at least of analytical quality.

Statistical analysis

All data are expressed as mean ± SD. The data were analyzed using a two-factor analysis of variance (ANOVA) with compound (AAs or catecholamines) and treatment (sham or NX) as sources of variation, and with repeated measures (RM) on the AAs factor. Significant main effects and interactions, with significance defined as $p < 0.05$, were probed using a post-hoc Fisher LSD test.

Results

NX mice develop biochemical changes compatible with RF showing clearly elevated plasma levels of CTN and urea and reduced creatinine clearance (CTN_{Cl}) when compared to the sham-operated mice (Table 1).

Table 2 represents the different plasma concentrations of AAs in the three experimental groups. Two-way RM-ANOVA shows a significant effect of the interaction treatment on plasma AA pattern ($F_{46,589} = 2.45$; $p < 0.001$). Pairwise comparison by Fisher LSD test shows that particularly plasma arginine ($p < 0.0001$), methionine ($p = 0.01$) and citrulline ($p = 0.002$) levels are significantly higher in NX group C compared to group B and controls. Taurine ($p < 0.05$), asparagine ($p = 0.003$), glutamine ($p = 0.02$), α-aminobutyric acid ($p = 0.02$) and proline ($p = 0.03$) are significantly increased in NX group B compared to controls and group C. Serine plasma concentration however was significantly decreased ($p < 0.02$) in group C compared to group B and controls. The other AA concentrations either remain unchanged or change minimally in a non-significant manner. It is noteworthy that more NEAAs undergo significant changes in plasma than EAAs.

Table 1. Biological parameters in sham-operated and nephrectomized mice 10 days post-surgery

Groups	A (n = 11)	B (n = 10)	C (n = 10)
P_{CTN} (μM)	21.3 ± 3.19	23.5 ± 2.93	35.3 ± 6.20***^{ooo}
P_{urea} (mM)	10.2 ± 2.63	12.6 ± 2.62	26.2 ± 9.83***^{ooo}
CTN_{Cl} (ml/min)	0.556 ± 0.204	0.526 ± 0.073	0.332 ± 0.091**^{oo}

A sham-operated group, *B* 50% nephrectomized group, *C* 75% nephrectomized group, *P* plasma, *CTN* creatinine, CTN_{Cl} creatinine clearance. Values are means ± SD with n = number of animals.
*$p < 0{,}05$, **$p < 0{,}01$, ***$p < 0{,}001$ versus group A; ^{o}p 0,05, $^{oo}p < 0{,}01$, $^{ooo}p < 0{,}001$ versus group B.

Table 2. Plasma levels of amino-acids in sham-operated and nephrectomized mice 10 days post-surgery

	A (n = 11)	B (n = 10)	C (n = 10)
Non-essential AA			
Taurine	311 ± 55.6	357 ± 122*	322 ± 99.4
Aspartic acid	6.33 ± 1.83	7.73 ± 3.49	6.53 ± 1.96
Serine	92.1 ± 23.1	93.6 ± 15.6	68.9 ± 14.9*^{o}
Asparagine	28.4 ± 5.11	46.2 ± 20.5**	25.7 ± 4.10^{oo}
Glutamic acid	35.7 ± 14.4	54.4 ± 30.5	31.8 ± 10.1
Glutamine	437 ± 65.5	566 ± 147**	466 ± 33.4^{o}
Glycine	188 ± 53.9	208 ± 27.5	201 ± 27.4
Alanine	344 ± 53.3	340 ± 78	331 ± 46.7
Citrulline	56.9 ± 12.3	69.2 ± 16.4	89.1 ± 23.6***^{o}
Ornithine	124 ± 30.1	135 ± 61	98.7 ± 33.3
Proline	83.2 ± 10.6	111 ± 37*	89.2 ± 13.7
Essential AA			
Arginine	18.8 ± 13.6	41.5 ± 14.6**	54.2 ± 24.4***
Threonine	117 ± 23.1	117 ± 31.2	91.9 ± 21.6
Valine	188 ± 24.8	169 ± 42.9	162 ± 44.9
Methionine	51.1 ± 16.2	84.9 ± 28.7*	94.6 ± 44.1**
Isoleucine	67.2 ± 13.7	66.8 ± 15.6	67.7 ± 22.7
Leucine	115 ± 23	112 ± 25	102 ± 30
Lysine	254 ± 49.7	253 ± 44	241 ± 30.1
Histidine	57 ± 6.55	64.9 ± 6.14	58.4 ± 10.9
Phenylalanine	61.8 ± 12.2	75.6 ± 26.2	69.9 ± 15.5
Tryptophan	70.5 ± 16.4	57.0 ± 13.8	60.2 ± 26.7
Semi-essential AA			
Tyrosine	63.4 ± 16.1	49.4 ± 9.29	54.9 ± 11.1
Cystine	<DL	<DL	<DL
Other AA			
Ethanolamine	<DL	<DL	<DL
3-CH3-Histidine	6.57 ± 1.19	5.57 ± 2.54	7.99 ± 2.77
α-amino-Butyric acid	4.55 ± 1.78	8.92 ± 5.38**	4.69 ± 1.29^{o}
Cystathionine	1.71 ± 0.389	2.01 ± 0.475	2.32 ± 0.254

Concentrations are expressed as μmol/l except for urea as mmol/l. Results are means ± SD.
*$p < 0{,}05$, **$p < 0{,}01$, ***$p < 0{,}001$ versus group A; ^{o}p 0,05, $^{oo}p < 0{,}01$ versus group B.

In urine (Table 3), the two-way RM-ANOVA detects a significant effect of treatment on AA pattern ($F_{50,696} = 12.3$; $p < 0.001$). The pairwise comparison by Fisher LSD test reveals significant increases in urinary elimination of some AAs. Most striking significant increases are found for alanine, citrulline and methionine in both NX groups compared to controls. A significant decrease in urinary excretion of glycine and ethanolamine is observed in both NX groups whereas urinary asparagine ($p < 0.04$) is only decreased in group C. Proline ($p = 0.01$) and cystathionine ($p < 0.001$) are significantly increased in group B compared to the control group and are unchanged in group C. Other AAs are either unchanged or under the

Table 3. Urine levels of amino-acids in sham-operated and nephrectomized mice 10 days post-surgery

	A (n = 11)	B (n = 11)	C (n = 10)
Non-essential AA			
Serine	0.576 ± 0.236	0.496 ± 0.069	0.732 ± 0.144*^{oo}
Asparagine	0.395 ± 0.134	0.473 ± 0.077	0.294 ± 0.109*^{oo}
Glutamic acid	0.436 ± 0.158	0.42 ± 0.179	0.365 ± 0.167
Glutamine	0.522 ± 0.148	0.628 ± 0.121	0.535 ± 0.188
Glycine	0.977 ± 0.223	0.723 ± 0.149**	0.71 ± 0.118**
Alanine	0.699 ± 0.243	0.867 ± 0.186*	1.21 ± 0.362***^{oo}
Citrulline	0.059 ± 0.032	0.097 ± 0.033**	0.09 ± 0.026*
Ornithine	0.671 ± 0.261	0.634 ± 0.15	0.561 ± 0.152
Proline	0.399 ± 0.143	0.568 ± 0.094**	0.468 ± 0.11
Essential AA			
Arginine	0.292 ± 0.075	0.279 ± 0.084	0.261 ± 0.073
Threonine	0.586 ± 0.262	0.535 ± 0.054	0.592 ± 0.121
Valine	0.334 ± 0.07	0.372 ± 0.107	0.362 ± 0.068
Methionine	0.912 ± 0.402	1.58 ± 0.225**	1.66 ± 0.61***
Isoleucine	0.116 ± 0.048	0.127 ± 0.035	0.147 ± 0.05
Leucine	0.365 ± 0.161	0.365 ± 0.087	0.347 ± 0.089
Lysine	0.471 ± 0.164	0.456 ± 0.061	0.465 ± 0.102
Histidine	0.148 ± 0.034	0.155 ± 0.021	0.148 ± 0.041
Phenylalanine	0.157 ± 0.059	0.114 ± 0.045	0.162 ± 0.041
Tryptophan	<DL	<DL	<DL
Semi-essential AA			
Tyrosine	0.206 ± 0.097	0.252 ± 0.074	0.339 ± 0.157*
Cystine	0.189 ± 0.057	0.245 ± 0.12	0.245 ± 0.068
Homocystine	0.185 ± 0.057	0.221 ± 0.07	0.196 ± 0.041
Other AA			
Ethanolamine	1.94 ± 0.615	1.36 ± 0.338**	0.688 ± 0.307***^{oo}
1-CH3-Histidine	0.293 ± 0.087	0.248 ± 0.07	0.227 ± 0.085
3-CH3-Histidine	0.342 ± 0.114	0.375 ± 0.049	0.477 ± 0.083**^{o}
Carnosine	<DL	<DL	<DL
Cystathionine	0.134 ± 0.054	0.226 ± 0.035***	0.157 ± 0.065^{oo}
α-amino-Adipinic acid	<DL	<DL	<DL
α-amino-Butyric acid	0.065 ± 0.029	0.069 ± 0.032	0.067 ± 0.049

Concentrations are expressed as μmol/24 h except for urea as mmol/24 h. Results are means ± SD.
*$p < 0{,}05$, **$p < 0{,}01$, ***$p < 0{,}001$ versus group A; ^{o}p 0,05, $^{oo}p < 0{,}01$ versus group B.

detection limit. Herein also, we observe that there are more NEAAs than EAAs with significantly changed urinary excretion.

In brain tissue, two-way RM-ANOVA shows a significant effect of interaction treatment on the different AAs ($F_{48,679} = 1.59$; $p = 0.008$) (Table 4) and the BAs and its catabolites ($F_{10,145} = 2.79$; $p = 0.004$) (Table 5), 10 days post-surgery. Concentrations of many essential and other AAs are increased in group C as cystathionine ($p = 0.001$), isoleucine ($p = 0.003$), leucine ($p = 0.01$), phenylalanine ($p < 0.0001$), β-alanine ($p < 0.0001$) and 3-methylhistidine ($p = 0.02$). Methionine and arginine are enhanced in both NX

Table 4. Brain levels of amino-acids in sham-operated and nephrectomized mice 10 days post-surgery

	A (n = 11)	B (n = 11)	C (n = 10)
Non-essential AA			
Taurine	9,921 ± 1,006	10,048 ± 1,231	9,622 ± 819*^{o}
Aspartate	3,129 ± 389	3,305 ± 326	3,527 ± 218*
Threonine	438 ± 91	501 ± 48	497 ± 67
Serine	1,437 ± 156	1,313 ± 107*	1,499 ± 111^{oo}
Glutamate	10,535 ± 478	11,126 ± 677*	10,762 ± 637^{o}
Glutamine	5,175 ± 452	5,807 ± 268**	5,489 ± 658*^{o}
Glycine	975 ± 139	1,025 ± 90.5	1,076 ± 140
Alanine	943 ± 66.7	1,047 ± 97.2*	883 ± 135^{ooo}
Citrulline	<DL	<DL	<DL
Ornithine	10.3 ± 6.09	8.64 ± 1.65	14.2 ± 7.03
Proline	<DL	<DL	<DL
Hydroxyproline	<DL	<DL	<DL
Essential AA			
Arginine	127 ± 16.3	153 ± 19*	171 ± 39***
Valine	121 ± 20.5	136 ± 14.3	139 ± 23.9
Methionine	5.35 ± 1.79	9.08 ± 3.01***	10.8 ± 1.89***
Isoleucine	45.2 ± 2.85	43.9 ± 4.04	52.5 ± 8.68**^{oo}
Leucine	86.4 ± 6.15	81.7 ± 6.96	99.8 ± 21.8*^{oo}
Lysine	263 ± 27.7	274 ± 24.6	254 ± 42
Histidine	90.9 ± 8.28	88.5 ± 10.1	97.6 ± 19.1
Phenylalanine	60.1 ± 7.47	62.4 ± 5.39	78.3 ± 15***^{ooo}
Tryptophan	<DL	<DL	<DL
Semi-essential AA			
Tyrosine	64.7 ± 20.9	78.2 ± 21.7	79.4 ± 20.5
Cystine	8.31 ± 2.44	8.60 ± 3.20	12.1 ± 6.60
Other AA			
Ethanolamine	422 ± 25.8	435 ± 78.3	443 ± 89.1
3-CH3-Histidine	4.21 ± 1.55	3.78 ± 0.759	5.44 ± 0.937*^{oo}
Carnosine	61.9 ± 7.36	69.9 ± 11.2	65.8 ± 8.14
γ-amino-Butyric acid	3,453 ± 249	3,643 ± 440	3,441 ± 312
β-Alanine	66.4 ± 3.39	62.1 ± 7.73	77.5 ± 8.74**^{ooo}
α-amino-Buteryric acid	<DL	<DL	<DL
Cystathionine	9.26 ± 2.32	8.60 ± 1.53	12.6 ± 3.05**^{ooo}

Concentrations are expressed as nmol/g tissue. Results are the means ± SD.
*$p < 0{,}05$, **$p < 0{,}01$, ***$p < 0{,}001$ versus group A; ^{o}p 0,05, $^{oo}p < 0{,}01$, $^{ooo}p < 0{,}001$ versus group B.

groups. Levels of glutamine ($p = 0.01$) and alanine ($p = 0.003$) are particularly increased in brain of group B whereas serine is significantly decreased ($p < 0.01$) compared to controls and group C. In group B animals, NEAA brain levels appear to change more than EAA levels, whereas in group C, EAA brain levels seem to be affected more. Some BAs and their metabolites are slightly but significantly changed in group C. Norepinephrine (NE), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) are increased compared to controls whereas 5-HT is decreased compared to group B.

Table 5. Brain levels of biogenic amines and catabolites in sham-operated and nephrectomized mice 10 days postsurgery

	A (n = 11)	B (n = 12)	C (n = 9)
NE	646 ± 181	667 ± 246	853 ± 179*
E	<DL	<DL	<DL
DOPAC	328 ± 162	481 ± 133	648 ± 269*
5HIAA	603 ± 88	582 ± 135	653 ± 180
DA	563 ± 258	603 ± 186	555 ± 244
HVA	476 ± 205	583 ± 127	651 ± 142*
5HT	629 ± 156	687 ± 113	502 ± 171°

NE norepinephrine, *E* epinephrine, *DOPAC* 3,4-dihydroxyphenylacetic acid, *5HIAA* 5-hydroxyindolacetic acid, *DA* dopamine, *HVA* homovanilic acid, *5HT* 5-hydroxytryptamine (serotonin). Concentrations are expressed as ng/g tissue. Values are means ± SD.
*$p < 0{,}05$ versus group A; °$p < 0{,}05$ versus group B.

Discussion

RF causes changes in plasma AAs which are difficult to evaluate because of the unusual dietary factors which prevail in advanced renal disease. It was found that EAAs are lowered in uremia when protein intake is substantially restricted (McGale et al., 1972). These changes rather reflect starvation and protein depletion than the course of uremia.

In this study, we investigated changes of AAs and BAs at an early stage of RF in an animal model in order to detect specific AA and BA alterations related to the reduction of functional renal mass. Alterations in plasma, urine and brain AA concentrations occur early, independently of the degree (~50% and ~75% NX) of RF. Many of the NEAAs undergo changes in plasma and urine in both NX groups when compared to the sham-operated group. This may mean that NEAA metabolism is more severely affected by the reduction of functional renal mass than EAA metabolism. Altered levels of NEAAs could result from changes in either AA reabsorption capacity (Perez et al., 1981), degradation of proteins or synthesis in the liver (Nakasaki et al., 1993).

Plasma levels of serine, an AA nearly exclusively synthesized in the kidney, decreased in group C. This might be a consequence of renal mass reduction and/or decreased conversion of glycine to serine (Pitts, 1970). The levels of citrulline and arginine, two AAs involved in the urea cycle, are elevated in plasma of both NX groups, which is in agreement with earlier reports in uremic patients and rats (Swendseid et al., 1978; Fürst et al., 1980; Levillain et al., 1997). Increased levels of citrulline in both plasma and urine may be caused by decreased conversion of citrulline to arginine (Swendseid et al., 1975) or decreased activity of kidney enzymes (Chan et al., 1974). Altered hepatic and/or intestinal uptake and release of citrulline (Windmueller et al., 1981), arginine (Hurwitz and Kretchmer, 1986) and other AAs like

methionine, lysine and glycine (Sterner et al., 1982) may have contributed to their changed levels.

The induced RF altered normal functioning of the kidney, and consequently changed filtration and excretion of AAs. The proximal tubule, principal site of nephron AA handling (Leslie and Vaughan, 1986; Levillain et al., 1993), is very vulnerable (Dobyan, 1985). Kidney failure and diminished tubular AA reabsorption may have caused the higher excretion of some AAs observed here (Betts and Green, 1977; Perez et al., 1980, 1981; Nadvornikova et al., 1978). Increased alanine excretion in the NX groups (especially in group C) might be explained by muscle proteolysis, as alanine is a major end-product of the degradation of AAs in tissues (Ishikawa et al., 1972). Another explanation could be an alteration in alanine and serine uptake since they share a common transport system in the kidney (Dobyan, 1985).

In this study, there was a significant difference in brain content of several AAs in the NX groups. Some of these differences may have been due to enzymatic alterations in the brain of NX mice. Increase in synthetic enzyme may have enhanced e.g. brain arginine concentration. Inhibition of nitric oxide synthase (NOS), which catalyses the transformation of arginine to nitric oxide (NO) and citrulline, might also explain the elevated arginine concentrations. Asymmetric dimethylarginine (ADMA), a compound found in normal brain tissue (Ogawa et al., 1987; Ueno et al., 1992), was found to be increased in plasma of uremic patients (Marescau et al., 1997; MacAllister et al., 1996) and rats (unpublished data), and is considered as a potent inhibitor of NOS (Vallance et al., 1992; Yu et al., 1994). Furthermore, sulfur AA levels (methionine and cystathionine) are enhanced in brain of both NX groups (particularly in group C). This might have been due to vitamin B_6 deficiency as a consequence of low food intake in the NX mice. The requirement of vitamin B_6 depend to some extend on the protein content of the diet. Moreover, studies indicated vitamin B_6 deficiency in uremic patients (Stone et al., 1975) causing an accumulation of cystathionine in the brain (Hope, 1964).

RF also had an effect on cerebral AA and BA concentrations as revealed in our data. As AAs are involved in brain biochemistry, structure and neurotransmitter function, their altered metabolism may lead to neurological disturbances. When concentrations of plasma AAs are altered, the transport across the blood brain barrier may be affected as well (Freeman et al., 1962; Jeppsson et al., 1982). Free AAs are very important for central nervous function since some of them are direct precursors of BAs. In accordance with other reports (Jeppsson et al., 1982; Kikuchi et al., 1984), we found the levels of phenylalanine and tyrosine, two precursors of the catecholamines, to be increased in brain of both NX groups. DOPAC, the primary DA metabolite, often reflects the amounts of DA released and reuptaked by nerve endings and dendrites (Korf et al., 1976). In our uremic model, enhanced DA levels may have induced elevated DOPAC and HVA concentrations, respectively.

Tryptophan, a precursor of 5-HT, is supplied from protein breakdown and diet. In uremia, some authors found a decrease in brain 5-HT (Siassi et al., 1977a), while others found an increase in 5-HT levels (Sullivan et al., 1980; Ksiazek et al., 1982). The decreased plasma tryptophan levels observed here,

may have resulted from decreased food intake in the NX mice (Fernstrom, 1978) or from alterations in the common transport system of tryptophan, phenylalanine, leucine, isoleucine, tyrosine, valine and methionine (Fernstrom, 1972). It was shown that phenylalanine inhibits tryptophan uptake into the brain (Blasberg and Lajtha, 1965; McKean et al., 1968). We observed increased phenylalanine levels in brain of NX mice. The reduced 5-HT synthesis was either due to a suppression of tryptophan transport into the brain, or an inhibition of tryptophan hydroxylase. On the other hand, increased MAO activity may explain the decreased brain 5-HT content observed in uremic patients (Tam et al., 1975; Siassi et al., 1977b).

In conclusion, the present study demonstrates early alterations in plasma, urine and brain AA profiles as a consequence of RF. Depending on the degree of RF, specific AAs are influenced. A clear understanding of AA profiles in normal renal function, and in different degrees of RF could help to establish efficient therapy (e.g. protein-restricted diet in dialyzed patients). If not restored at an early stage of RF, AA disturbances could be one of the factors contributing to the development of metabolic and/or neurological complications in uremic patients.

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