Amino Acids (2001) 21: 401–415

Amino
Acids

The effect of high protein diet on urea and guanidino compound levels in renal insufficient mice

M. Al Banchaabouchi[1], **B. Marescau**[1], **R. D'Hooge**[1], and **P. P. De Deyn**[1,2]

[1]Department of Neurology, Laboratory of Neurochemistry and Behaviour at the Born-Bunge Foundation, University of Antwerp, Belgium
[2]Department of Neurology at Middelheim General Hospital, Antwerp, Belgium

Accepted January 9, 2001

Summary. Nephrectomy in mice provokes a decrease in creatinine clearance (CTN_{Cl}) and an increase in urea and specific guanidino compound (GC) concentrations in blood and other tissues. Our purpose was to investigate the influence of high protein diet (HPD) on CTN_{Cl}, urea and GC levels in NX mice. Mice were nephrectomized or sham-operated and subdivided in groups to study five diet conditions. At the end of each experiment, 10 days and 30 days postsurgery, urine and blood were collected for determination of urea and GCs, including creatinine. HPD resulted in significantly higher CTN_{Cl} values in sham-operated mice than those observed in mice under normal protein diet, 10 days as well as 30 days postnephrectomy. HPD induced significant increases in plasma urea, guanidinosuccinic acid, argininic acid and α-keto-δ-guanidinovaleric acid concentration 10 days postsurgery but not 30 days postsurgery. HPD coincided with significantly higher excretion of urea, guanidinosuccinic acid, α-keto-δ-guanidinovaleric acid, creatine, argininic acid and γ-guanidinobutyric acid in sham-operated and nephrectomized mice 10 days postsurgery. Our results show that HPD induces supplementary (to nephrectomy) increases of urea and GCs in the early postsurgery period but not in the later phase.

Keywords: Amino acids – High protein diet – Guanidino compound levels – Nephrectomy – Mice – Renal insufficiency

Introduction

Guanidino compounds (GCs) are metabolic products of proteins and amino acids characterized by a guanidino group (Robin and Marescau, 1985). For some of the GCs, like arginine and creatine, their important role in nitrogen metabolism is well known whereas for others, like guanidinosuccinic acid (GSA), α-N-acetylarginine (NAA) and methylguanidine (MG) it is not their

physiological role but rather their toxicity which has been demonstrated (May et al., 1996; Vanholder, 1997, 1999). Various GCs accumulate in renal failure (RF) (De Deyn et al., 1987, 1995; Marescau et al., 1997) and are believed to be responsible for some uremic symptoms (De Deyn et al., 2001). In addition, some of these compounds, e.g., GSA and MG, were also demonstrated to be increased in plasma and urine in normal and uremic patients with high protein intake (Cohen, 1970; Kopple et al., 1977; Orita et al., 1981). It was suggested that in uremia, high protein intake provided increased nitrogen end-products (Cohen, 1970). Excessive protein intake is believed to increase uremic toxicity by stimulating formation of uremic toxins from the metabolism of proteins. Therefore, it may be useful to follow up GC metabolism in uremic patients when given specific nutritional regimens.

Excess protein intake also influences renal hemodynamics both in man and animals with normal or reduced renal function (Hostetter et al., 1981; Brenner et al., 1982; Bergström et al., 1985). Moreover, protein intake has been proposed to modulate glomerular permeability in single kidney patients (Coppo et al., 1988) and in the rat renal ablation model (Hostetter et al., 1986). However, it was shown in dogs that protein loading does not have an adverse effect on renal function and induces only minimal changes in renal histology (Robertson et al., 1986; Bovée, 1991). It is generally accepted that restricting dietary protein intake can ameliorate some of the clinical signs of uremia by reducing nitrogen containing wastes and possibly by slowing down renal failure progression. For this reason, low protein diet is usually prescribed to patients with chronic renal failure. However, it is still debated as to when to restrict protein intake, how much protein is needed, and in how far protein restriction will delay the progression of renal disease, since the effect of protein restriction on the rate of renal function loss remains uncertain (Maroni et al., 1997). Until now, no studies have investigated the influence of HPD on the whole spectrum of the GCs found in mammalia. Neither were studies done on the potentially differential impact of early and late onset HPD in renal insufficient mice. Therefore, we evaluated these issues by studying the changes in GC levels with NPD and HPD at two different periods post-nephrectomy. Renal insufficient mice were compared to sham-operated ones, 10 days and one month postsurgery.

Materials and methods

Animals and surgery

Adult male mice (2-month-old Swiss-Webster × C57BL hybrids, bred under our laboratory facilities) weighing around 20–26 g were used. Before surgery, all the mice were initially fed normal protein diet (NPD), containing 20% casein, for ten days. We considered two groups: a sham-operated group (SH) and a nephrectomized group (NX). Renal failure was induced by a ligature technique as described previously (Al Banchaabouchi et al., 1998). Mice were anesthetized with pentobarbital 60 mg/kg body weight, i.p.). Via a small bilateral dorsal flank incision to expose the kidneys, SH group underwent a sham operation. In NX group, in a single step procedure, the right kidney was removed after the contralateral kidney was partially infarcted by ligation of the

anterior renal artery branch. Mice were put in their cages and were allowed tap water and food *ad libitum* during the study.

According to the local ethics committee approval of the protocol for the study was obtained.

Protocol of the experiments

Before operation, mice were maintained on NPD, containing 20% casein, during 10 days. Following the surgical procedure, animals were subdivided in groups to study five diet conditions: (1) a group consisting of Sham (n = 9) and NX (n = 10) animals under NPD examined 10 days postsurgery, (2) a group consisting of Sham (n = 9) and NX (n = 9) animals under HPD (containing 36% casein) examined 10 days postsurgery, (3) a group consisting of Sham (n = 6) and NX (n = 5) animals under NPD examined 30 days postsurgery, (4) a group consisting of Sham (n = 7) and NX (n = 7) animals under HPD examined 30 days postsurgery, and finally (5) a group consisting of Sham (n = 6) and NX (n = 6) animals under NPD for 10 days followed by HPD for a period of 20 days.

At the end of each experiment, we collected urine and sacrified the animals for blood analysis.

Diet

Mice were fed 2 commercial diets (purshased from Pavan Service Carfil Quality, Oud-Turnhout, Belgium) one with protein content of 20% (adequate to supprot growth) and the second with 36% (as a high protein diet). The difference in casein content was substituted by glucose, so the diets were isocaloric. The two diets had the same content of energy (3748 Kcal, gross), vitamins and mineral mix.

Collection and preparation of plasma and urine samples

From each experimental animal, urine was collected in a metabolic cage (Tecniplast, Garracla S., Buguggiate (VA), Italy), adapted for mice, over a 48 hours period. On the 11th day, mice were anesthetized with pentobarbital (60 mg/kg body weight, i.p.) and, from the orbital venous plexus, samples of blood were collected into heparinized vacutainer tubes. Plasma was obtained after centrifugation at 700 × g at 6°C for 10 min. A portion of 5 μL was taken for urea determination. The remaining plasma was used for GC analysis.

GCs analyzed were: α-keto-δ-guanidinovaleric acid (**GVA**); guanidinosuccinic acid (**GSA**); creatine (**CT**); guanidinoacetic acid (**GAA**); α-N-acetylarginine (**NAA**), argininic acid (**AA**), β-guanidinopropionic acid (**GPA**); creatinine (**CTN**); γ-guanidinobutyric acid (**GBA**); arginine (**Arg**), homoarginine (**HA**); guanidine (**G**) and methylguanidine (**MG**).

Urea and guanidino compound analysis

Urea nitrogen was determined with diacetylmonoxime as described by Ceriotti (1971). For GC analysis, plasma and urine were deproteinized by mixing equal volume of a 200 g/L trichloroacetic acid solution with plasma or urine. The acidic protein complexes were precipitated in a Beckman microfuge E (Beckman Instruments, Inc, Palo Alto, California 94304, USA) at 15 850 × g for 10 min. The supernatant was used for GC 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). The GCs were separated over a cation exchange column using

sodium citrate buffers and were detected with the fluorescence ninhydrin method. Standard GCs were purchased from Sigma Chemical Company (St. Louis, MO), creatine and creatinine from Merck (Darmstadt, Germany). α-Keto-δ-guanidinovaleric acid was synthesized enzymatically as described earlier (Marescau et al., 1992). All other chemicals used were obtained from Merck and were of analytical grade.

Statistical analysis

Serial measurements were analyzed for treatment effects, diet effects, time effects and interactions, using a three-way analysis of variance (ANOVA). A pairwise comparison (Fischer PLSD test) was performed only when ANOVA was significant, with significance defined as $P < 0.05$. All data are expressed as mean $\pm$ SD.

Results

Body weight, urinary volume and CTN_{Cl}

Table 1 shows the biological changes which occur after NX and HPD. A three-way ANOVA is used to assess the changes in mean body weight (BW), urinary volume (V_{ur}) and CTN_{Cl} with surgery (SH or NX), diet (NPD or HPD) and duration (10 or 30 days) as sources of variation. Analysis showed that BW was significantly affected by surgery ($F_{1,45} = 10.6$; $P = 0.002$) and duration ($F_{1,45} = 7.9$; $P = 0.007$); V_{ur} by surgery ($F_{1,51} = 81.8$; $P < 0.001$) and diet ($F_{1,51} = 17.0$; $P < 0.001$); and CTN_{Cl} by surgery ($F_{1,52} = 55.5$; $P < 0.001$), diet ($F_{1,52} = 12.2$; $P < 0.001$) and duration ($F_{1,52} = 5.1$; $P = 0.028$). Interaction between surgery, diet and duration significantly affected BW ($F_{1,45} = 5.8$; $P = 0.02$), which was reflected by significantly lower BW in NX mice in the 10 days HPD and 30 days NPD groups, but not in the other groups. Results in Table 1 demonstrate that only SH mice receiving HPD have significantly higher BW ($P < 0.05$).

It is shown in Table 1 how V_{ur} is mostly considerably higher in the NX animals, however, HPD results in a greater increase in V_{ur} by approximately 2-fold in both SH and NX groups, at 10 days postsurgery. In addition, the interactions surgery $\times$ diet ($F_{1,51} = 7.0$; $P = 0.011$) and surgery $\times$ duration ($F_{1,51} = 4.7$; $P = 0.034$) had significant effects on V_{ur} indicating that the effects of nephrectomy are influenced by diet as well as duration. Indeed, changes of V_{ur} appear to be more pronounced under HPD and at 10 days post-nephrectomy. NX induces a decrease in CTN_{Cl} of mice under NPD and HPD. However, HPD itself induces CTN_{Cl} values in SH mice which are significantly higher (1.3-fold) than those observed in mice under NPD, 10 days as well as 30 days postsurgery (Table 1). Both treated groups manifest a significant difference between early and late onset of HPD in CTN_{Cl} ($P < 0.05$; one way ANOVA). The value of CTN_{Cl} is greater when HPD is given at the day of NX compared to when this is administered 10 days later.

Plasma GC findings

Variation in plasma GC levels at 10 and 30 days postsurgery and with the two diets in SH and NX mice are shown in Table 2. As revealed by three way

Table 1. Biological data in sham-operated and nephrectomized (at the age of 2 months) mice at 10 and 30 days postsurgery

		BW (g)		V_{ur} (mL/48h)		CTN_{Cl} (mL/min)	
	Period on and type of diet	SH	NX	SH	NX	SH	NX
10 days PS	10 days NPD	22 ± 1.8 (9)	22 ± 2.4 (9)	1.92 ± 0.84	7.15 ± 1.8*	0.58 ± 0.08	0.38 ± 0.08*
	10 days HPD	25 ± 1.9 (9)♦	22 ± 2.7* (9)	3.28 ± 0.76♦	15 ± 3.7*♦	0.76 ± 0.16♦	0.41 ± 0.14*
	30 days NPD	26 ± 2.8 (6)⊛	22 ± 2.3* (5)	3.17 ± 0.69⊛	7.32 ± 3.13*	0.65 ± 0.18	0.43 ± 0.12*
30 days PS	10 days NPD + 20 days HPD	25 ± 2.6 (7)	23 ± 3.2 (7)	2.97 ± 1.06	11 ± 2.9*	0.54 ± 0.09	0.36 ± 0.08*
	30 days HPD	25 ± 1.09 (6)	24 ± 2 (6)	3.9 ± 1.9	10 ± 5.8⊛	0.83 ± 0.20♦$	0.53 ± 0.07*⊛$

SH sham-operated mice; *NX* ± 70% nephrectomized mice; *NPD* normal protein diet (180.18 g casein/kg); *HPD* high protein diet (359.179 g casein/kg); *BW* body weight; V_{ur} urine volume; CTN_{Cl} creatinine clearance; *PS* postsurgery. Results are means ± SD. * $P < 0.05$ NX versus SH. Asteriks represent statistical significance between SH and NX groups. ♦ $P < 0.05$, NPD versus HPD; ⊛ $P < 0.05$, 10 versus 30 days postsurgery; $ $P < 0.05$, early versus late onset of HPD.

Table 2. Plasma concentrations of urea and GCs in sham-operated and nephrectomized (at the age of 2 month) mice under NPD and HPD intake, during 10 days and 30 days starting from the day of their operation

	10 days postsurgery				30 days postsurgery					
	10 days NPD		10 days HPD		30 days NPD		10 days NPD + 20 days HPD		30 days HPD	
	SH (n = 9)	NX (n = 10)	SH (n = 9)	NX (n = 9)	SH (n = 6)	NX (n = 5)	SH (n = 7)	NX (n = 7)	SH (n = 6)	NX (n = 6)
GVA	0.12 ± 0.05	0.323 ± 0.118*	0.082 ± 0.017	0.53 ± 0.37*♦	0.08 ± 0.05	0.198 ± 0.138	0.17 ± 0.06	0.23 ± 0.13	0.103 ± 0.054$	0.196 ± 0.109⁎
GSA	0.132 ± 0.019	0.68 ± 0.27*	0.19 ± 0.06♦	2.13 ± 1.71*♦	0.137 ± 0.019	1.08 ± 0.77*	0.19 ± 0.05	0.59 ± 0.13*	0.166 ± 0.024	0.68 ± 0.28*⁎
CT	117 ± 23	109 ± 5.9	115 ± 37	123 ± 51.4	109 ± 29	112 ± 42.9	117 ± 25	133 ± 29	98 ± 28	121 ± 23.4
GAA	1.18 ± 0.26	0.98 ± 0.19	1.28 ± 0.29	1.19 ± 0.31	1.27 ± 0.29	0.840 ± 0.155*	1.35 ± 0.21	1.09 ± 0.29	1.24 ± 0.28	0.816 ± 0.197*⁎$
NAA	<0.007	0.12 ± 0.06	<0.007	0.15 ± 0.11	<0.007	<0.007	<0.007	ND	<0.007	0.08 ± 0.04
AA	0.092 ± 0.027	0.24 ± 0.08*	0.07 ± 0.036	0.37 ± 0.15*♦	0.047 ± 0.016	0.128 ± 0.085*	0.11 ± 0.07	0.19 ± 0.09	0.08 ± 0.04	0.2 ± 0**
GPA	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007
CTN	16.8 ± 1.6	26 ± 2.3*	14.5 ± 2.4♦	24.8 ± 8.7*	16.6 ± 2.05	23.5 ± 3.4*	17.6 ± 2.8	24.1 ± 3.4*	13.7 ± 1.8♦$	19.6 ± 2.3
GBA	<0.007 − 0.05	<0.007	<0.007 − 0.06	0.113 ± 0.063	<0.007	<0.007	<0.007	<0.007	0.027 ± 0.012	<0.007
Arg	55 ± 45	59 ± 21	32 ± 16	45 ± 27	58 ± 32	58 ± 27	49 ± 19	56.5 ± 23.4	46.3 ± 14.6	42 ± 17
HA	0.57 ± 0.26	0.37 ± 0.09*	0.34 ± 0.09♦	0.39 ± 0.14	0.30 ± 0.13*	0.224 ± 0.079*	0.32 ± 0.13	0.27 ± 0.09	0.28 ± 0.08	0.187 ± 0.052*⁎
G	<0.03 − 0.12	0.18 ± 0.06	0.17 ± 0.04	0.144 ± 0.061	0.113 ± 0.052	0.235 ± 0.009*	0.11 ± 0.05	0.085 ± 0.026	0.143 ± 0.082	0.117 ± 0.029♦
MG	<0.01	<0.01 − 0.04	<0.01	0.053 ± 0.015	<0.01	<0.01 − 0.04	<0.01	ND	<0.01 − 0.03	<0.01 − 0.03
Urea	9.1 ± 1.2	21.2 ± 5.2*	10 ± 3	40 ± 2*♦	8.07 ± 0.828	20.4 ± 8.5*	10.2 ± 2.2	19.3 ± 5.9*	10.7 ± 1.9	22.5 ± 6.9*⁎

GVA α-keto-δ-guanidinovaleric acid; *GSA* guanidinosuccinic acid; *CT* creatine; *GAA* guanidinoacetic acid; *NAA* α-N-acetylarginine; *AA* argininic acid; *GPA* β-guanidinopropionic acid; *CTN* creatinine; *GBA* γ-guanidinobutyric acid; *Arg* arginine; *HA* homoarginine; *G* Guanidine; *MG* methylguanidine.

Results are means ± SD. Concentrations are expressed as μmol/L except for urea as mmol/L. * $P < 0.05$, asteriks represent significant difference between SH and NX. ♦ $P < 0.05$, NPD versus HPD; ⁎ $P < 0.05$, 10 versus 30 days postsurgery; $ $P < 0.05$, early versus late onset of HPD.

ANOVA, GCs affected by NX are GVA ($F_{1,52} = 24.1$; $P < 0.001$), GSA ($F_{1,52} = 26.7$; $P < 0.001$), GAA ($F_{1,52} = 17.1$; $P < 0.001$), AA ($F_{1,46} = 58.7$; $P < 0.001$), CTN ($F_{1,52} = 55.5$; $P < 0.001$), HA ($F_{1,52} = 4.37$; $P < 0.05$), G ($F_{1,41} = 4.41$; $P < 0.05$) and urea ($F_{1,52} = 37.2$; $P < 0.001$). NX induces increased plasma levels of urea, GSA, GVA, AA and CTN. The GAA and HA levels seem to be decreased in plasma after NX, those of CT and Arg to be changed.

The three way ANOVA also showed that the type of diet (NPD or HPD) significantly influenced levels of AA ($F_{1,46} = 6.57$; $P = 0.014$), CTN ($F_{1,52} = 5.37$; $P = 0.025$), Arg ($F_{1,52} = 4.87$; $P = 0.032$) and urea ($F_{1,52} = 5.25$; $P = 0.026$). NX and SH mice fed HPD differed in their plasma GC levels from those fed NPD. Mainly at 10 days postsurgery, an increase occurs in urea and AA levels (~2-fold and 1.6-fold respectively, $P < 0.05$, one way ANOVA) in NX mice only. At 10 and 30 days postsurgery, SH groups manifested an unexplained decrease in CTN levels with HPD versus NPD ($P < 0.05$). The same tendency is seen at 30 days postsurgery for the NX groups (t-test; $P = 0.048$).

The effect of duration has only minor effects observed in the case of GVA ($F_{1,52} = 7.46$; $P = 0.009$), AA ($F_{1,46} = 13.9$; $P < 0.001$) and HA ($F_{1,52} = 20.7$; $P < 0.001$) affected. NX groups showed more pronounced levels of GVA and AA ($P < 0.05$, one way ANOVA) at 10 days than at 30 days postsurgery. HA levels are significanlty lower ($P < 0.05$) at 30 days than at 10 days postsurgery in NX.

A significant interaction is found between treatment, diet and duration on GSA ($F_{1,52} = 5.66$; $P = 0.021$) where the highest increase in GSA levels are seen in NX mice fed with HPD at 10 days postsurgery.

The interaction treatment × duration has a significant effect on GVA ($F_{1,52} = 6.44$; $P = 0.014$), GAA ($F_{1,52} = 4.22$; $P < 0.05$) and AA ($F_{1,46} = 8.38$; $P = 0.01$). For GVA, levels are more increased at 10 days postsurgery in the NX mice with the two diets. GAA levels in the NX mice are significantly decreased with early onset when compared to late onset of HPD.

Interaction diet × treatment significantly affected levels of G ($F_{1,46} = 13.5$; $P < 0.001$) and AA ($F_{1,46} = 5.32$; $P = 0.026$). Comparing HPD to NPD, only NX mice presented lower levels of G at 30 days postsurgery ($P < 0.001$, one way ANOVA) and increased levels of AA (1.6-fold; $P < 0.05$) at 10 days postsurgery.

The impact of early and late onset of HPD on GC metabolism was compared in both SH and NX groups. At 30 days postsurgery, no spectacular differences were found. Changes were minimal as observed on the higher levels of GVA and CTN (in SH group) and GAA only (in NX groups) with late onset of HPD.

Urine GC findings

The amount of GCs excreted in urine are presented in Table 3. The increase or decrease in GC elimination depends mainly on treatment (sham or NX) and diet (NPD or HPD). Changes in excretion due to NX are observed for

Table 3. Urinary excretion of urea and GCs in sham-operated and nephrectomized (at the age of 2 month) mice under NPD and HPD intake, during 10 days and 30 days starting from the day of their operation

	10 days postsurgery				30 days postsurgery					
	10 days NPD		10 days HPD		30 days NPD		10 days NPD + 20 days HPD		30 days HPD	
	SH (n = 9)	NX (n = 10)	SH (n = 9)	NX (n = 9)	SH (n = 6)	NX (n = 5)	SH (n = 7)	NX (n = 7)	SH (n = 6)	NX (n = 6)
GVA	0.11 ± 0.03	0.071 ± 0.019*	0.174 ± 0.049♦	0.153 ± 0.089♦	0.119 ± 0.023	0.08 ± 0.03*	0.197 ± 0.064	0.14 ± 0.03*	0.16 ± 0.04	0.12 ± 0.05
GSA	0.05 ± 0.01	0.088 ± 0.019*	0.108 ± 0.016♦	0.261 ± 0.063*♦	0.067 ± 0.011	0.137 ± 0.029*	0.089 ± 0.017	0.19 ± 0.05*	0.124 ± 0.026$♦	0.21 ± 0.08*♦✠
CT	6.31 ± 2.36	5.31 ± 2.16	9.23 ± 1.99♦	9.46 ± 2.94♦	3.54 ± 1.27✠	3.03 ± 1.74	6.10 ± 2.18	6.38 ± 2.22	7.9 ± 1.9♦	8.4 ± 4.6♦
GAA	2.43 ± 0.44	1.28 ± 0.33*	2.77 ± 0.48	1.02 ± 0.69*	2.75 ± 0.47	1.10 ± 0.44*	1.96 ± 0.23	0.981 ± 0.415*	2.5 ± 0.4$	1.20 ± 0.23*
NAA	0.041 ± 0.016	0.046 ± 0.011	0.047 ± 0.006	0.055 ± 0.009*	0.059 ± 0.018	0.053 ± 0.015	0.049 ± 0.013	0.045 ± 0.008	0.042 ± 0.008	0.047 ± 0.012
AA	0.068 ± 0.014	0.061 ± 0.021	0.115 ± 0.018♦	0.126 ± 0.015♦	0.057 ± 0.014	0.051 ± 0.015	0.13 ± 0.03	0.116 ± 0.028	0.122 ± 0.024♦	0.109 ± 0.031♦
GPA	0.004 ± 0.001	0.004 ± 0.001	0.004 ± 0.001	0.004 ± 0.001	0.005 ± 0.001	0.004 ± 0.015	0.004 ± 0.001	0.003 ± 0.001	0.004 ± 0.001	0.003 ± 0.001
CTN	13.9 ± 2.1	13.6 ± 2.5	15.5 ± 2.5	13.3 ± 2.52	15.4 ± 3.8	14.2 ± 2.7	13.4 ± 1.6	12.1 ± 1.25	16.1 ± 2.8	14.8 ± 2.1
GBA	0.053 ± 0.017	0.039 ± 0.009*	0.061 ± 0.008	0.08 ± 0.03♦	0.042 ± 0.005	0.035 ± 0.018	0.071 ± 0.011	0.062 ± 0.022	0.068 ± 0.018♦	0.05 ± 0.01*✠
Arg	0.334 ± 0.194	0.102 ± 0.072*	0.181 ± 0.056♦	0.344 ± 0.207♦	0.15 ± 0.02✠	0.129 ± 0.070	0.154 ± 0.035	0.101 ± 0.046*	0.16 ± 0.05	0.16 ± 0.06✠
HA	<DL − 0.007	<DL − 0.008	<DL − 0.008	<DL − 0.081	0.009 ± 0.002	<DL	<DL − 0.008	<DL	0.007 ± 0.001	<DL
G	0.114 ± 0.047	0.12 ± 0.04	0.102 ± 0.018	0.098 ± 0.010	0.203 ± 0.084✠	0.18 ± 0.04✠	0.087 ± 0.033	0.078 ± 0.028	0.106 ± 0.017♦	0.100 ± 0.029♦
MG	0.017 ± 0.005	0.027 ± 0.006*	0.019 ± 0.003	0.025 ± 0.005*	0.018 ± 0.004	0.021 ± 0.004	0.019 ± 0.007	0.022 ± 0.005	0.019 ± 0.007	0.019 ± 0.005✠
Urea	2.39 ± 0.52	2.36 ± 0.54	4.49 ± 0.55♦	4.92 ± 0.43♦	2.49 ± 0.72	2.29 ± 0.62	3.91 ± 0.69	4.21 ± 0.46	4.3 ± 0.39♦	4.31 ± 0.95♦

Results are means ± SD. Concentrations are expressed as μmol/24 h except for urea as mmol/24 h. * $P < 0.05$, asteriks represent significant difference between SH and NX. ♦ $P < 0.05$, NPD versus HPD; ✠ $P < 0.05$, 10 versus 30 days postsurgery; $ $P < 0.05$, early versus late onset of HPD.

GVA ($F_{1,52} = 7.68$; $P = 0.008$), GSA ($F_{1,52} = 70.6$; $P < 0.001$), GAA ($F_{1,52} = 142$; $P < 0.001$) and MG ($F_{1,52} = 12.3$; $P = 0.001$). Urinary excretion of GVA and GAA decreases, whereas those of GSA and MG increase as compared to the SH groups.

Effects of diet on concentration are observed for GVA ($F_{1,52} = 20.3$; $P < 0.001$), GSA ($F_{1,52} = 75.9$; $P < 0.001$), CT ($F_{1,52} = 39$; $P < 0.001$), AA ($F_{1,52} = 129$; $P < 0.001$), GBA ($F_{1,50} = 21.6$; $P < 0.001$), G ($F_{1,52} = 23.5$; $P < 0.001$) and urea ($F_{1,52} = 184$; $P < 0.001$). Indeed, with HPD, in both SH and NX mice, higher excretion levels of GVA, GSA, CT, AA, GBA and urea are found except for G ($P < 0.05$). An effect of duration on CT ($F_{1,52} = 7.83$; $P = 0.007$), GBA ($F_{1,50} = 4.78$; $P = 0.033$), Arg ($F_{1,45} = 7$; $P = 0.011$) and G excretion ($F_{1,52} = 12.6$; $P < 0.001$) is also observed. The amount of GBA and Arg excreted after 30 days HPD by NX mice is significantly lower than the amounts excreted after 10 days HPD. Also SH mice excrete lower CT and Arg amount after 30 days NPD, however excretion of G is higher compared to the amount excreted after 10 days NPD.

An interaction effect diet $\times$ treatment is found for GSA ($F_{1,52} = 9.94$; $P = 0.003$) and Arg ($F_{1,50} = 9.35$; $P = 0.004$). Indeed, the influence of HPD, at 10 days postsurgery, on GSA and Arg is greater in the NX group than in the SH group. Moreover, an interaction effect diet $\times$ duration significantly influences GSA ($F_{1,52} = 6.66$; $P = 0.013$), NAA ($F_{1,52} = 8.77$; $P = 0.005$) and G ($F_{1,52} = 10.8$; $P = 0.002$) excretion. Comparing HPD to NPD, both SH and NX groups show up to 3-fold increased excretion of GSA 10 days postsurgery and approximately 2-fold decrease in G 30 days postsurgery.

Significant interaction between the three factors diet, duration and treatment is found for GSA ($F_{1,52} = 6.71$; $P = 0.012$), GBA ($F_{1,50} = 6.63$; $P = 0.013$) and Arg ($F_{1,45} = 7.76$; $P = 0.008$). In NX mice, HPD feeding has a greater effect on GSA ($P < 0.05$) and Arg ($P < 0.05$) excretion mainly at 10 days postsurgery, whereas for GBA ($P < 0.05$) it is at 30 days postsurgery only. A decrease in Arg excretion is found at 30 days postsurgery with NPD feeding in SH mice ($P < 0.05$) and with HPD feeding in NX mice ($P < 0.05$). Finally, an interaction effect between treatment and duration is detected for MG ($F_{1,52} = 4.89$; $P = 0.031$). MG excretion increases mainly 10 days postnephrectomy ($P < 0.05$).

At 30 days postsurgery, a difference between early and late onset of HPD was observed only in the SH groups where higher GSA and GAA excretion were found with early onset of HPD compared to the late onset.

Discussion

Protein intake and reduction of renal mass influenced renal function and GC metabolism in our experimental mice. With the regard to renal function, CTN_{Cl} decreased in the experimental animals after NX at 10 and 30 days postsurgery under both diets. Furthermore, CTN_{Cl} was higher in SH mice which started HPD from the day of surgery. Increased CTN_{Cl} in SH mice suggest that the glomerular filtration capacity of the kidney was increased

from 0.58 ± 0.08 to 0.83 ± 0.20 mL/min. This enhancement correspond to the so-called renal function reserve (Bosh et al., 1983). Our statistical results studying the duration effect of HPD show in the NX group a significant increase of CTN_{Cl} 30 days post-nephrectomy. This might be explained by a maximal function of all available nephrons at 10 days postsurgery corresponding with the acute phase of renal failure (Bosch et al., 1983), while at 30 days postsurgery a partial restoration of the renal functional reserve is established.

The increase of CTN_{Cl} in mice fed 36% casein diet indicates that kidney function still present a renal functional reserve, a capacity found as well in normal as in some uremic patients (Bosch et al., 1983, 1984; Dhaene et al., 1987; Rugiu et al., 1987). This finding may have important clinical implications. Consequently to the acute effect of NX, the remnant kidney displays a hypertrophy and hyperfiltration which function is more or less adapted some time postNX. Some authors found that after ingestion of HPD, uremic patients and animals had increased CTN_{Cl} (Hostetter et al., 1986; Tufro et al., 1991) whereas others found it to be decreased (Kenner et al., 1985) or unchanged (Perelstein et al., 1990). However, Bosch et al. (1983) and Chan et al. (1988) even showed increased glomerular filtration rate after HPD intake. Some studies in man and animals demonstrate that HPD results in glomerular hyperfiltration that consequently contributes to further deterioration of renal function (Kleinknecht et al., 1979; Brenner et al., 1982; Alvestrand et al., 1983). This suggested that protein restriction may also ameliorate glomerular hyperfiltration, reduce GC levels and delay renal disease progression in patients with CRF (Ando et al., 1979). In view of the results presented here, protein restriction may have to start as soon as possible in order to reduce the ultimate damage to the remaining viable nephrons.

Under normal conditions, when food is available ad libitum, individual meals are smaller but can be more frequent, and total food and protein consumption is substantially increased (Tucker et al., 1976). Despite the fact that our experimental mice were given a HPD, ad libitum, our uremic mice showed only a tendency towards increased BW. Obviously, reduction of renal mass induced an anorexia-like syndrome. It is known that uremic patients tend to lose their appetite and reduce protein and energy intakes spontaneously (Ikizler et al., 1995), and emaciate (Mitch, 1998). The potential disadvantages of protein restriction may include protein malnutrition characterized by weight loss, reduction in muscle mass, and reduced albumin. As known in uremia, patients tend to be in negative nitrogen balance contributing to higher requirements of protein and amino acids (Fürst et al., 1980; Young and Pellett, 1987). Although treatment with protein restriction can alleviate uremic symptoms and improve patient's health (Ando et al., 1979), prolonged severe protein restriction may, in itself, produce malnutrition. Thus, the crucial issue in RF remains to find an equilibrium between restriction of protein intake, attenuate the deterioration of renal function (Gansevoort et al., 1997), and negative nitrogen balance due to malnutrition. Consistent with previous findings (Harri and Brockway, 1985; Hashimoto et al., 1996), we have observed that NX resulted in an increase in V_{ur}, which was

even more pronounced (2-fold) 10 days postsurgery in mice fed HPD. This shows that HPD stimulates water intake and increases urine output.

Furthermore, irrespective of the type of diet, changes of GC concentrations in plasma and urine were primarily altered by NX implying that impairment of renal function has a major effect on GC metabolism. This observation was found earlier in uremic patients (Cohen, 1968; De Deyn et al., 1987, 1995; Suzuki, 1992; Marescau et al., 1997) and experimental animals (Cohen, 1970; Levillain et al., 1995; Al Banchaabouchi, 1998). Nevertheless, the levels of GC concentrations in body fluids showed different responses to NPD/HPD and feeding period. This was manifested by raised plasma concentrations of some GCs, e.g., GVA, GSA, AA, GBA, MG and urea with HPD feeding at day 10 postsurgery versus lower concentration at day 30 postsurgery. Also higher urinary excretion of some GCs (e.g., GSA, AA) and urea with HPD was observed, especially 10 days postsurgery.

Notably, both in normal subjects and uremic patients, other authors also found that urinary GSA and urea excretion increased with HPD (Cohen, 1970; Sawynok and Dawborn, 1975) and decreased with low protein intake (Kopple et al., 1977; Ando et al., 1979). They suggested that this increase might reflect increased GSA production whereas for urea, an end-product of protein metabolism, its high level in blood is related directly to protein intake and to renal excretory capacity. Thus, the tremendous increase of plasma GSA (11-fold), AA (5-fold) and urea (4-fold) concentrations after HPD intake, in NX group as compared to their controls, suggests that HPD further turns the metabolic flow towards a series of GCs. Starved mice have been shown to present decreased levels of GCs suggesting that GC levels principally depend on exogenous nitrogen (Shindo et al., 1986), and that most of the GCs could be synthesized directly or indirectly from Arg (Shindo and Mori, 1983; Natelson, 1984). HPD might cause, especially at the early stage after NX, worsening of the uremic symptoms and damage to the kidney (Tufro et al., 1991). The functional status of the remnant kidney and its ability to continue to function adequately with HPD has to be taken into careful consideration. Onset of HPD in the early stage of NX may also have negative consequences on other organs than kidney, such as the central nervous system. It has been demonstrated that high increase of GSA, GBA and MG causes vascular and neurological disturbances such as epilepsy (Giovannetti, 1973; Mori, 1983; Shiraga, 1991; D'Hooge et al., 1992, 1994; Segarra G, 1999). Urea might be involved in the development of atherosclerosis associated with renal failure, since in vitro studies showed that high levels of urea may reduce nitric oxide synthesis (Moeslinger et al., 1999). Remarkably, 30 days postsurgery and under HPD, significanlty lower plasma concentration (urea, GSA, GVA, GAA, AA and HA) and urinary excretion (GSA, GBA, Arg and MG) are observed in NX mice. An adaptive response to the pathophysiological condition (NX), changes in diet (HPD) and duration may explain this decrease. In this context the tendency of the BW to increase in NX mice after 30 days postsurgery compared to 10 days HPD can be noted. The importance of protein intake is directly related to the net degradation rate of protein and some amino acids, this may indicate lower turnover rate in the

urea cycle and protein metabolism (Goodship et al., 1990). As our experiments showed, late onset of HPD in NX mice did not cause major changes neither in the biological parameters nor in urea nor GC metabolism.

In conclusion, our results show firstly that, HPD induces an addition early increase in candidate uremic toxins in plasma and urine (10 days) after NX. Secondly, after 30 days HPD the supplementary increases are no more observed in plasma of NX mice; the urinary excretion is even lower. Finally, our study also reveals how late onset of HPD slightly influence GC metabolism. Thus especially in the early stages of NX, HPD leads to supplementary increases of candidate uremic toxins in plasma and urine of our animals.

Acknowledgments

We thank Ilse Possemiers for her technical work.

References

Al Banchaabouchi M, Marescau B, D'Hooge R, Van Marck E, Van Daele A, Levillain O, De Deyn PP (1998) Biochemical and histopathological changes in nephrectomized mice. Metabolism 47: 355–361

Alvestrand A, Ahlberg M, Bergstrom J (1983) Retardation of the progression or renal insufficiency in patients treated with low-protein diets. Kidney Int [Suppl 16]: S268–S272

Ando A, Orita Y, Nakata K, Tsubakihara Y, Takamitsu Y, Ueda N, Yanase M, Abe H (1979) Effect of low protein diet and surplus of essential amino acids on the serum concentration and the urinary excretion of methylguanidine and guanidinosuccinic acid in chronic renal failure. Nephron 24: 161–169

Bergström J, Ahlberg M, Alvestrand A (1985) Influence of protein intake on renal hemodynamics and plasma hormone concentrations in normal subjects. Acta Med Scand 217: 189–196

Bosch JP, Saccaggi A, Lauer A, Ronco C, Belledonne M, Glabman S (1983) Renal functional reserve in humans. Effect of protein intake on glomerular filtration rate. Am J Med 75: 943–950

Bosch JP, Lauer A, Glabman S (1984) Short-term protein loading in the assessment of renal function. Am J Med 77: 873–879

Bovée KC (1991) Influence of dietary protein on renal function in dogs. J Nutr 121: S128–S139

Brenner BM, Meyer TW, Hostetter TH (1982) Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. N Engl J Med 307: 652–659

Ceriotti G (1971) Ultramicrodetermination of plasma urea by reaction with diacetylmonoxime-antipyrine without deproteinization. Clin Chem 17: 400–402

Chan AY, Cheng ML, Keil LC, Myers BD (1988) Functional response of healthy and diseased glomeruli to a large, protein-rich meal. J Clin Invest 81: 245–254

Cohen BD (1970) Guanidinosuccinic acid in uremia. Arch Intern Med 126: 846–850

Cohen BD, Stein IM, Bonas JE (1968) Guanidinosuccinic aciduria in uremia. A possible alternate pathway for urea synthesis. Am J Med 45: 63–68

Coppo R, Amore A, Roccatello D, Martina G, Rollino C, Basolo B, Piccoli G (1988) Microalbuminuria in single kidney patient: relationship with protein intake. Clin Nephrol 29: 219

De Deyn PP, Marescau B, Cuykens JJ, Van Grop L, Lowenthal A, De Potter WP (1987) Guanidino compounds in serum and cerebrospinal fluid of non dialyzed patients with renal insufficiency. Clin Chim Acta 167: 81–88

De Deyn PP, Marescau B, D'Hooge R, Possemiers I, Nagler J, Mahler C (1995) Guanidino compound levels in brain regions of non-dialyzed uremic patients. Neurochem Int 27: 227–237

De Deyn PP, D'Hooge R, Van Bogaert PP, Marescau B (2001) Endogenous guanidino compounds as uremic toxins. Kidney Int 59 [Suppl 78]: S77–83

Dhaene M, Sabot JP, Philippart Y, Doutrelepont JM, Vanherweghem JL (1987) Effects of acute protein loads of different sources on glomerular filtration rate. Kidney Int [Suppl 22]: S25–S28

D'Hooge R, Pei QY, Marescau B, De Deyn PP (1992) Convulsive action and toxicity of the uremic guanidino compounds: behavioral assessment and relation to brain concentration in adult mice. J Neurol Sci 112: 96–105

D'Hooge R, Pei YQ, Marescau B, De Deyn PP (1994) Ontogenetic differences in convulsive action and cerebral uptake of uremic guanidino compounds in juvenile mice. Neurochem Int 24: 215–220

Fürst P, Alvestrand A, Bergström J (1980) Effects of nutrition and catabolic stress on intracellular amino acid pools in uremia. Am J Clin Nutr 33: 1387–1395

Gansevoort RT, de Zeeuw D, de Jong PE (1997) Effect of protein restriction on deterioration of kidney function. Ned Tijdschr Geneeskd 141: 2106–2110

Giovannetti S, Balestri PL, Barsotti G (1973) Methylguanidine in uremia. Arch Intern Med 131: 709–713

Goodship TH, Mitch WE, Hoerr RA, Wagner DA, Steinman TI, Young VR (1990) Adaptation to low-protein diets in renal failure: leucine turnover and nitrogen balance. J Am Soc Nephrol 1: 66–75

Harri M, Brockway JM (1985) Effect of dietary protein concentration and ambient temperature on the energy, protein and water metabolism of the rat. Br J Nutr 53: 363–372

Hashimoto M, Funaba M, Abe M, Ohshima S (1996) Effect of chronic high protein intake on magnesium, calcium, and phosphorus balance in growing cats. Exp Anim 45: 63–70

Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM (1981) Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. Am J Physiol 9: F85–F93

Hostetter TH, Meyer TW, Rennke HG, Brenner BM (1986) Chronic effects of dietary protein in the rat with intact and reduced renal mass. Kidney Int 30: 509

Ikizler TA, Greene JH, Wingard RL, Parker RA, Hakim RM (1995) Spontaneous dietary protein intake during progression of chronic renal failure. J Am Soc Nephrol 6: 1386–1391

Kenner CH, Evan AP, Blomgren P, Aronoff GR, Luft FC (1985) Effect of protein intake on renal function and structure in partially nephrectomized rats. Kidney Int 27: 739–750

Kleinknecht C, Salusky I, Broyer M, Gubler MC (1979) Effect of various protein diets on growth, renal function, and survival of uremic rats. Kidney Int 15: 534–541

Kopple JD, Gordon SI, Wang M, Swendseid ME (1977) Factors affecting serum and urinary guanidinosuccinic acid in normal and uremic subjects. J Lab Med 90: 303–311

Levillain O, Marescau B, De Deyn PP (1995) Guanidino compound metabolism in rats subjected to 20% and 90% nephrectomy. Kidney Int 47: 464–472

Marescau B, Deshumkh DR, Kockx M, Possemiers I, Qureshi IA, Wiechert P, De Deyn PP (1992) Guanidino compounds in serum, urine, liver, kidney and brain of man and some ureotelic animals. Metabolism 41: 526–532

Marescau B, Nagels G, Possemiers I, De Broe ME, Because I, Billiouw JM, Lornoy W, De Deyn PP (1997) Guanidino compounds in serum and urine of nondialyzed patients with chronic renal insufficiency. Metabolism 46: 1024–1031

Maroni BJ, Mitch WE (1997) Role of nutrition in prevention of the progression of renal disease. Annu Rev Nutr 17: 435–455

May RC, Mitch WE (1996) Pathophysiology of uremia. In: Brenner BM (ed) The kidney, 5th edn. Saunders, Philadelphia, pp 2148–2169

Mitch WE (1998) Mechanisms causing loss of lean body mass in kidney disease. Am J Clin Nutr 67: 359–366

Moeslinger T, Friedl R, Volf I, Brunner M, Baran H, Koller E, Spieckermann PG (1999) Urea induces macrophage proliferation by inhibition of inducible nitric oxide synthesis. Kidney Int 56: 581–588

Mori A (1983) Guanidino compounds and neurological disorders. Neuroscience 9: 149–157

Natelson S (1984) Metabolic relationship between urea and guanidino compounds as studied by automated fluorimetry of guanidino compounds in urine. Clin Chem 30: 252–258

Orita Y, Ando A, Tsubakihara Y, Mikami H, Kikuchi T, Nakata K, Abe H (1981) Tissue and blood cell concentration of methylguanidine in rats and patients with chronic renal failure. Nephron 27: 35–39

Perelstein EM, Grunfeld BG, Simsolo RB, Gimenez MI, Gianantonio CA (1990) Renal function reserve compared in haemolytic uraemic syndrome and single kidney. Arch Dis Child 65: 728–731

Robertson JL, Goldschmidt M, Kronfeld DS, Tomaszewski JE, Hill GS, Bovee KC (1986) Long-term renal responses to high dietary protein in dogs with 75% nephrectomy. Kidney Int 29/2: 511–519

Robin Y, Marescau B (1985) Natural guanidino compounds. In: Mori A, Cohen BD, Lowenthal A (eds) Guanidine: historical, biological, biochemical and clinical aspects of the naturally occuring guanidino compounds. Plenum Press, New York, pp 383–439

Rugiu C, Oldrizzi L, Maschio G (1987) Effects of an oral protein load on glomerular filtration rate in patients with solitary kidneys. Kidney Int [Suppl 22]: S29–S31

Sawynok J, Dawborn JK (1975) Plasma concentration and urinary excretion of guanidine derivatives in normal subjects and patients with renal failure. Clin Exp Pharmacol Physiol 2: 1–15

Segarra G, Medina P, Ballester RM, Lluch P, Aldasoro M, Vila JM, Lluch S, Pelligrino DA (1999) Effects of some guanidino compounds on human cerebral arteries. Stroke 30: 2206–2211

Shindo S, Mori A (1983) Metabolism of L-[amidino-^{15}N]-arginine to guandino compounds. In: Mori A, Cohen BD, Lowenthal A (eds) Guanidines compounds: historical, biological, biochemical, and clinical aspects of the naturally occurring guanidino. Plenum Press, New York London, pp 71–81

Shindo S, Watanabe Y, Mori A (1986) Effects of starvation of guanidino compounds in mice. Res Comm Chem Pathol Pharm 54: 73–83

Shiraga H, Watanabe Y, Mori A (1991) Guanidino compound levels in the serum of healthy adults and epileptic patients. Epilepsy Res 8: 142–148

Suzuki Y (1992) Guanidino compounds and aliphatic monoamines in acute and chronic renal failure. Jpn J Nephrol (Nippon Jinzo Gakkai Shi) 34: 1077–1085

Tucker SM, Mason RL, Beauchene RE (1976) Influence of diet and feed restriction on kidney function on aging male rats. J Gerontol 31: 264–270

Tufro A, Arrizurieta EE, Repetto H (1991) Renal functional reserve in children with a previous episode of haemolytic-uraemic syndrome. Pediatr Nephrol 5: 184–188

Young VR, Pellet L (1987) Protein intake and requirements with reference to diet and health. Am J Clin Nutr 45: 1323–1343

Vanholder R (1997) Uremic toxins. Adv Nephrol 26: 143–162
Vanholder R, De Smet R (1999) Pathophysiologic effects of uremic retention solutes. J Am Soc Nephrol 10: 1815–1823

Authors' address: Prof. Dr. Peter Paul De Deyn, Laboratory of Neurochemistry and Behaviour, Born-Bunge Foundation, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen, Belgium, Fax: +323 820 26 18, E-mail: ppdedeyn@uia.ua.ac.be

Received June 13, 2000