Biochemical and Histopathological Changes in Nephrectomized Mice

Mumna Al Banchaabouchi, Bart Marescau, Rudi D'Hooge, Eric Van Marck, Andre Van Daele, Olivier Levillain, and Peter Paul De Deyn

Renal failure is characterized by the retention of nitrogenous metabolites such as urea, creatinine (CTN) and other guanidino compounds (GCs), uric acid, and hippuric acid, which could be related to the clinical syndrome associated with renal insufficiency. A model of renal failure has been developed in male C57BL × Swiss-Webster mice using nephrectomy (NX) and/or arterial ligation. A sham group (group A) and two nephrectomized groups, group B (one kidney removed) and group C (one kidney removed and ligation of the contralateral anterior artery branch), were studied. Ten days postsurgery, morphological and functional indices of renal failure were investigated. Nephrectomized mice manifested features of renal failure like polyuria and wasting. CTN clearance (CTN_{Cl}) decreased by ±26% in group B and ±33% in group C as compared with the control values. Marked increases in the plasma concentration of guanidinosuccinic acid ([GSA] fourfold) and guanidine ([G] twofold) were observed in the experimental animals. CTN and α-keto-δ-guanidinovaleric acid (α-keto-δ-GVA) reached levels of, respectively, 1.5-fold and twofold those of controls. Urinary GSA excretion increased and guanidinoacetic acid (GAA) excretion decreased about twofold in group C. GSA increases (2.6-fold) were also observed in the brain in group C, in addition to a significant increase of G (2.5-fold) and γ-guanidinobutyric acid ([GBA] 1.5-fold). Finally, the extent of NX was found to be 45.2% in group B and 71.4% in group C. Light microscopy revealed an expansion and increase in cellularity of the mesangium of the glomeruli, particularly in group C. A significant correlation ($r = .574$, $P < .0001$) was found between CTN_{Cl} and the degree of NX as calculated from the remaining functional area. These data suggest that the model can be used as a tool for further pathophysiological and/or behavioral investigations of renal failure.

AMONG HUMAN DISORDERS, renal failure remains a serious health problem, and several animal models of renal failure have been developed to investigate various aspects of this syndrome.[1-4] Renal failure is characterized by the retention of nitrogenous metabolites such as urea, creatinine (CTN) and other guanidino compounds (GCs), uric acid, and hippuric acid, which could be related to the clinical syndrome associated with renal insufficiency.[5-8]

Although rats[9,10] and dogs[11,12] are frequently used as experimental animal models, some studies on chronic renal failure have used mice as well.[13,14] None of the studies on mice investigated GC levels, but some of the rat and dog studies mentioned these substances.[15-17] In the present study, we induced renal failure in mice by nephrectomy (NX) and/or arterial ligation and determined GC levels by cation-exchange chromatography with fluorescence detection. At the same time, histological changes on renal tissue were investigated.

The surgical method used here was used hitherto in rats[16] and dogs.[12] Hence, the purpose of the present study was to develop an experimental model of renal failure in mice, to demonstrate the reproducibility of renal failure using this technique, and also to determine biochemical changes by quantification of urea and GCs (including CTN) in the plasma, urine, and brain.

MATERIALS AND METHODS

Animals and Surgery

Young adult male mice (C57BL × Swiss-Webster cross-breed) weighing about 20 to 26 g were used. The experiments considered three groups, each with 14 animals. Two groups were nephrectomized, and the control group was sham-operated. A ligature technique was used to induce renal failure. In these mice, the renal artery proximal to the renal hile generally divides approximately at the middle into anterior and posterior branches. These two subdivisions ensure the supply of blood to approximately two thirds and one third, respectively. The mice were anesthetized with Hypnorm/midazolam (1 part Hypnorm [Janssen Pharmaceutica, Beerse, Belgium], 1 part Dormicum, and 2 parts water for injection, 4 mL/kg intraperitoneally).[18]

Via a small bilateral dorsal flank incision to expose the kidneys, group A mice underwent a sham operation. In group B, mice were nephrectomized: the renal artery and vein and the ureter were ligated together and cut, and the right kidney was then removed. In group C, the mice were also nephrectomized to approximately the same degree by removing the right kidney and ligating the anterior renal artery branch of the left kidney.

Animals were kept in 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 after the operation, the mice were housed in normal cages, and then they were kept in metabolic cages (Techniplast Garracla S., Buguggiate [VA], Italy) for urine collection over a 48-hour period.

Collection and Preparation of Plasma and Urine Samples

For all of the animals, urine was collected in metabolic cages adapted for mice. On day 11, after decapitation, blood was collected through a funnel in heparinized tubes. Plasma was obtained after centrifugation at 700 × *g* at 6°C for 10 minutes. A 5-μL portion was taken for urea determination, and the remaining plasma was used for GC analysis. For the determination of GCs, plasma and urine samples were deproteinized by mixing equal volumes of a 200-g/L trichloroacetic acid solution with plasma or urine. The proteins were centrifuged in a Beckman microfuge

From the Department of Medicine-Universitaire Instelling Antwerpen, Laboratory of Neurochemistry and Behavior, Born-Bunge Foundation, Department of Pathology, and Department of Neurology, Middelheim General Hospital, University of Antwerp, Antwerp, Belgium; and Faculté de Médecine Lyon R.T.H. Laënnic, Laboratoire de Physiopathologie Métabolique et Rénale, Lyon, France.

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Address reprint requests to Peter Paul De Deyn, MD, PhD, Laboratory of Neurochemistry and Behavior, Born-Bunge Foundation, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen, Belgium.

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Table 1. Biological Parameters in Sham-Operated and Renal Failure Mice 10 Days Postoperatively

Parameter	A		B		C	
	Mean ± SD	No.	Mean ± SD	No.	Mean ± SD	No.
Body weight (g)						
Day 0	20.7 ± 1.49	14	21.6 ± 1.91	14	21.3 ± 2.45	12
Day 10	21.4 ± 2.82	14	23.8 ± 2.02	14	18.3 ± 2.89*§	12
Urine volume (mL/24 h)	0.717 ± 0.317	14	0.984 ± 0.421	14	2.80 ± 0.941†§	12
CTN_{Cl} (mL/min)	0.480 ± 0.117	13	0.356 ± 0.091*	13	0.322 ± 0.137*	12
P urea (mmol/L)	7.97 ± 1.10	13	11.2 ± 2.17	13	22.5 ± 11.7†§	12
P CTN (μmol/L)	25.0 ± 4.37	14	34 ± 7.64*	14	35.3 ± 12.1*	12
Kidney mass (L + R, mg) day 10	364 ± 58.2	14	246 ± 27.9†	14	207 ± 45.5†‡	12

NOTE. Results were statistically analyzed by 1-factor ANOVA with Fisher's PLSD test.
Abbreviations: P, plasma; L, left; R, right (only for group A).
*$P < .01$, †$P < .001$: *v* group A.
‡$P < .05$, §$P < .001$: *v* group B.

(Beckman Instruments International, Geneva, Switzerland). The supernatant was used for GC determination.

Preparation of Brain Samples

The brains were removed and stored at −75°C until preparation for determination of GCs. At the time of analysis, the left hemisphere was homogenized in 1 mL water at 0°C with a "tissue tearor" (model 985; Biospec Products, Bartlesville, OK). The probe was washed immediately with 1 mL 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 minutes at 6°C), the clear supernatant was used for GC analysis.

GC Analysis

The concentration of GCs was determined using a Biotronic LC 5001 (Biotronik, Maintal, Germany) amino acid analyzer adapted for GC determination. GCs were separated over a cation-exchange column using sodium citrate buffers and detected with the ninhydrin fluorescence method as reported in detail.[19]

Urea nitrogen was determined with diacetylmonoxime as described by Ceriotti.[20]

Standard GCs were purchased from Sigma Chemical (St Louis, MO), and creatine and CTN from Merck (Darmstadt, Germany). α-Keto-δ-guanidinovaleric acid (α-Keto-δ-GVA) was synthesized enzymatically as described previously.[19] All other chemicals were obtained from Merck and were of analytical grade.

Microscopic Examination

The remnant kidney(s) of each mouse in all groups was removed, weighed, and placed in 10% Formol for histopathological examination. The kidneys were processed for light microscopy by embedding in paraffin and staining the sections with hematoxylin/eosin. Initially, the kidneys were transsected lengthwise, and both halves were cut at 5 μm thickness. Morphometric evaluation of the renal tissue was made by image analysis. Three images were obtained with a Dialux microscope (Leitz, Wetzlar, Germany) equipped with a 1× objective, a green, red, and blue filter of 540 nm, and a videocamera (CCD C27; MTI, Michigan City, MI) under standard conditions. The three images (green, red, and blue) were digitized with a Vidas Image Processing System (Kontron Elektronik, Eching, Germany). By mixing these three images, a static real-color image was obtained. Measurement of the net kidney area (without lumen) was performed with the standard image-processing and software of the Vidas system. The necrotic area was traced on the real-color display and calculated.

Statistics

Statistical analysis included one-way ANOVA with Fisher's protected least-significant difference (PLSD) test. A *P* value less than .05 was considered statistically significant. All data are presented as the mean ± SD.

Fig 1. Kidneys of groups A, B, and C mice. Sham-operated mouse, normal kidney (left). Kidney from a nephrectomized mouse in group B (middle), note hypertrophy; kidney from a nephrectomized mouse in group C with necrotic tissue in the upper part and the remaining viable tissue with hypertrophy in the lower part (right).

Table 2. Microscopic Findings in the Renal Tissue

Parameter	Group A (n = 14)	Group B (n = 14)	Group C (n = 13)
Net area (L + R, cm^2)	0.862 ± 0.108	0.472 ± 0.049†	0.360 ± 0.058*‡
Necrotic area (L + R, cm^2)	0	0	0.114 ± 0.045†‡
Functional area (L + R, cm^2)	0.862 ± 0.108	0.472 ± 0.049†	0.247 ± 0.047†‡

NOTE. Results are expressed as the mean ± SD and were statistically analyzed by 1-factor ANOVA with Fisher's PLSD. Net area is the surface of renal tissue without lumen.

Abbreviations: L, left; R, right (only for group A).

*$P < .01$, †$P < .001$: *v* group A.

‡$P < .001$ *v* group B.

RESULTS

General Observations

Biological parameters of the mice with varying degrees of NX are summarized in Table 1. On day 0 before surgery, the mean body weight of nephrectomized mice was not statistically different from that of sham-operated animals. Subsequent to the operation on day 10, a notable decrease in body weight was observed in group C compared with groups B and A. Polyuria with pale yellow urine due to a reduced renal concentrating ability was noted as a characteristic feature of renal failure in the nephrectomized mice. Group C produced the greatest volume of urine.

A significant difference in kidney weight was noted between the three groups. The total kidney mass (left and right) in group A at day 10 was 364 ± 58.2 mg, for an average of 182 mg for one kidney. In group B, the remaining kidney (left kidney) increased in weight by 35%. In group C, the remaining left kidney portion increased by 13.7%. These findings illustrate the compensatory hypertrophy of the remaining kidneys or kidney parts. In group C, the ischemic part of the kidney was necrotized, explaining the smaller increase in weight due to the residual viable tissue only (Fig 1).

Figure 1 illustrates that both nephrectomized groups responded identically despite the difference in the degree of renal injury induced by ligation, as shown by the morphology of the kidney. Hypertrophy was clearly marked in group B compared with group A at 10 days postsurgery, which also occurred in the remnant kidney tissue in group C (Fig 1).

Degree of NX

We measured the area of renal tissue sections in the control and nephrectomized groups. The percentage of NX for groups B and C was estimated by calculating the percentage of functional area (remnant viable tissue) with respect to the net area in group A (day 10). The actual percentage of functional area on day 10 was calculated to be decreased, with ±45.2% in group B and ±71.4% in group C as compared with control group A (Table 2).

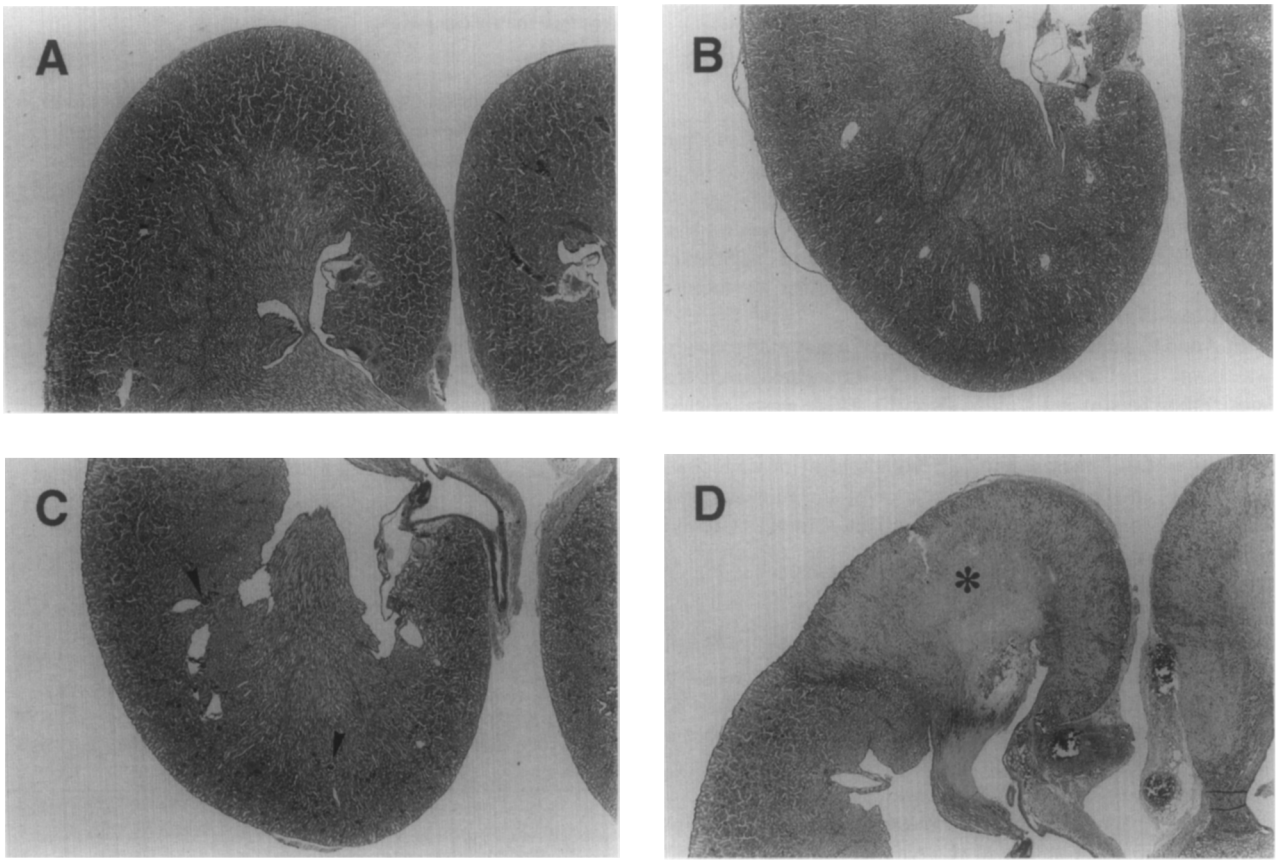

Fig 2. Kidney sections on light microscopy (hematoxylin and eosin, original magnification ×10.5). (A) Sham-operated mouse, normal histology; (B) renal section of group B, no visible alterations; (C) remnant viable renal tissue of group C, enlargement of the glomeruli (arrows); (D) necrotic renal tissue (*) of group C.

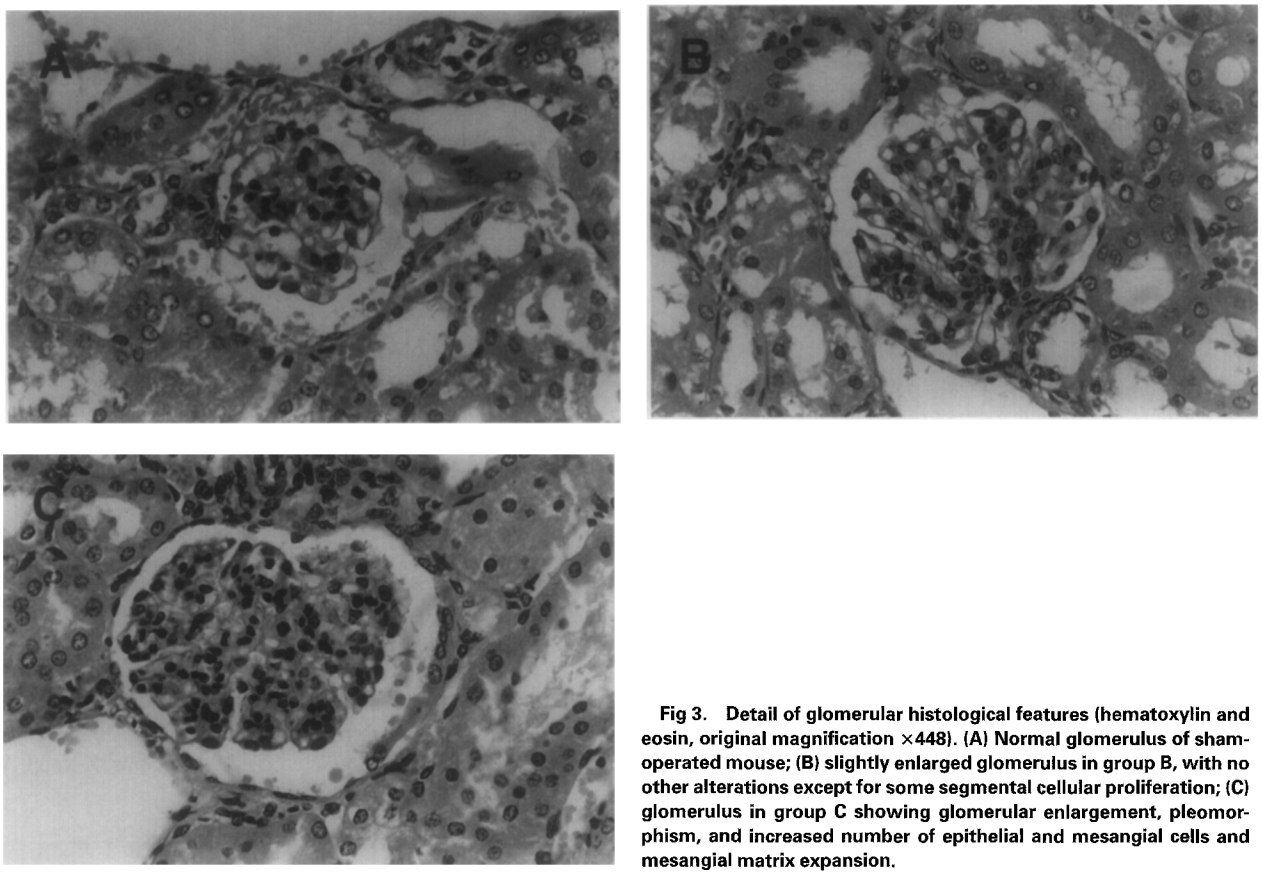

Fig 3. Detail of glomerular histological features (hematoxylin and eosin, original magnification ×448). (A) Normal glomerulus of sham-operated mouse; (B) slightly enlarged glomerulus in group B, with no other alterations except for some segmental cellular proliferation; (C) glomerulus in group C showing glomerular enlargement, pleomorphism, and increased number of epithelial and mesangial cells and mesangial matrix expansion.

Histopathological Findings in Renal Tissue

Histological sections under light-microscopic examination showed no renal abnormalities in sham-operated animals (Figs 2A and 3A), whereas in groups B and C the renal tissue did display morphological alterations. In group B, except for a slight widening of Bowman's space and some segmental cellular proliferation, there were no glomerular or tubular alterations (Figs 2B and 3B).

In group C, on the other hand, the necrotic part of the kidney was easily identified (Fig 2D) and well delineated from the surrounding healthy renal tissue (Fig 2C). There was a striking hypertrophy of the glomeruli, with a marked increase in the cellularity of the mesangium, a pleomorphism of epithelial, endothelial, and mesangial glomerular cells, and an increase of the mesangial matrix (Fig 3C). The tubuli, interstitial tissue, and vessels did not show any abnormalities.

Determination of GCs

Plasma. Ten days postsurgery, GC concentrations differed between the three groups. The most severely nephrectomized mice (group C) showed a more marked change in plasma GC concentrations than the mice of group B: most GCs showed a significant increment in group C in comparison to group A and also to group B, except for CTN, which was increased in a comparable manner in both nephrectomized groups. For instance, guanidinosuccinic acid (GSA) and γ-guanidinobutyric acid (GBA) were increased fourfold compared with the values in group A, and α-keto-δ-GVA and guanidine (G) were increased twofold. Smaller changes in creatine, arginine, and homoarginine levels were observed, as they increased in group B but decreased or remained unchanged in group C. Some GCs such as methylguanidine (MG) were below or near the detection limit in the control group but increased in the nephrectomized groups after 10 days (Table 3).

Urine. Excretion of GCs in the urine of nephrectomized

Table 3. Plasma GC Concentrations in Sham-Operated and Renal Failure Mice 10 Days Postoperatively

GC	Group A	Group B	Group C
α-Keto-δ-GVA	0.237 ± 0.080	0.34 ± 0.063*	0.532 ± 0.147‡¶
GSA	0.182 ± 0.043	0.294 ± 0.055*	0.737 ± 0.236‡¶
Creatine	304 ± 76.8	371 ± 48.5*	293 ± 93.9§
GAA	2.13 ± 0.54	2.39 ± 0.645	1.77 ± 0.380§
α-*N*-Acetylarginine	<0.015-0.1	<0.015-0.53	<0.015-0.31
Argininic acid	<0.015-0.014	<0.015-0.12	0.24 ± 0.12
β-Guanidinopropionic acid	<0.013-0.04	<0.013-0.07	<0.013-0.09
CTN	25.0 ± 4.37	34.0 ± 7.64†	35.3 ± 12.1†
GBA	0.35 ± 0.28	0.627 ± 0.213	1.47 ± 0.706‡¶
Arginine	123 ± 50.2	216 ± 108*	191 ± 98.2
Homoarginine	0.385 ± 0.090	0.438 ± 0.087	0.314 ± 0.060*¶
G	0.291 ± 0.072	0.331 ± 0.070	0.463 ± 0.156‡‖
MG	<0.02-0.08	0.105 ± 0.041	0.14 ± 0.046

NOTE. Results are the mean ± SD (or range) for 14, 14, and 12 mice, respectively, in groups A, B, and C. Concentrations are expressed as μmol/L. Results were statistically analyzed by 1-factor ANOVA with Fisher's PLSD test.

*$P < .05$, †$P < .01$, ‡$P < .001$: *v* group A.

§$P < .05$, ‖$P < .01$, ¶$P < .001$: *v* group B.

Table 4. Urinary Excretion Levels of GCs and Urea in Sham-Operated and Renal Failure Mice 10 Days Postoperatively

Parameter	Group A	Group B	Group C
α-Keto-δ-GVA	0.150 ± 0.039	0.135 ± 0.029	0.121 ± 0.036*
GSA	0.057 ± 0.013	0.079 ± 0.015*	0.106 ± 0.037†‡
Creatine	7.45 ± 1.76	7.57 ± 2.31	6.91 ± 1.89
GAA	2.04 ± 0.55	1.61 ± 0.390*	0.861 ± 0.368†§
α-*N*-Acetylarginine	0.039 ± 0.010	0.044 ± 0.010	0.044 ± 0.010
Argininic acid	0.048 ± 0.011	0.051 ± 0.013	0.055 ± 0.016
β-Guanidinopropionic acid	0.010 ± 0.002	0.012 ± 0.002	0.013 ± 0.010
CTN	16.8 ± 2.84	16.97 ± 3.13	14.9 ± 3.04
GBA	0.788 ± 0.328	0.932 ± 0.256	0.924 ± 0.261
Arginine	0.297 ± 0.147	0.321 ± 0.112	0.279 ± 0.108
Homoarginine	<DL	<DL	<DL
G	0.266 ± 0.068	0.318 ± 0.096	0.275 ± 0.069
MG	0.089 ± 0.023	0.153 ± 0.126*	0.101 ± 0.025
Urea	1.99 ± 0.413	2.31 ± 0.497	2.28 ± 0.467

NOTE. Results are the mean ± SD for 14, 14, and 12 mice, respectively, in groups A, B, and C. Concentrations are expressed as μmol/24 h except for urea (mmol/24 h). Results were statistically analyzed by 1-factor ANOVA with Fisher's PLSD test.

Abbreviation: DL, detection limit.

*$P < .05$, †$P < .001$: *v* group A.

‡$P < .01$, §$P < .001$: *v* group B.

animals did not change significantly, except for GSA, which increased significantly, and guanidinoacetic acid (GAA), which declined, compared with group A. CTN had the highest excretion, and it was in the same range in both the sham-operated and nephrectomized groups (Table 4).

Brain. High levels of GSA and G were found in group C; the increase was approximately threefold compared with levels in the sham-operated mice. On the other hand, a weak increase of GBA and arginine was observed in both nephrectomized groups. The other GCs did not show significant alterations (Table 5).

Table 5. Brain GC Levels in Sham-Operated and Renal Failure Mice 10 Days Postoperatively

GC	Group A	Group B	Group C
α-Keto-δ-GVA	1.19 ± 0.222	1.13 ± 0.080	1.24 ± 0.213
GSA	0.148 ± 0.037	0.198 ± 0.043	0.383 ± 0.128‡‖
Creatine	8,506 ± 1,025	8,103 ± 974	8,159 ± 979
GAA	8.60 ± 1.07	10.4 ± 1.11†	9.43 ± 1.82
α-*N*-Acetylarginine	1.08 ± 0.238	1.19 ± 0.367	1.03 ± 0.309
Argininic acid	2.79-0.480	2.70 ± 0.350	0.24 ± 0.12
β-Guanidinopropionic acid	<DL-0.390	<DL-0.26	<DL-0.32
CTN	240 ± 53.2	267 ± 27.6	262 ± 61.7
GBA	1.60 ± 0.225	1.89 ± 0.209	2.33 ± 0.885†
Arginine	151 ± 19.8	174 ± 21.3*	171 ± 28.1
Homoarginine	0.601 ± 0.138	0.570 ± 0.143	0.650 ± 0.268
G	1.23 ± 1.01	1.148 ± 0.602	3.14 ± 2.29†§
MG	2.34 ± 0.997	2.94 ± 0.818	2.40 ± 1.18

NOTE. Results are the mean ± SD for 11, 12, and 9 mice, respectively, in groups A, B, and C. Concentrations are expressed as nmol/g tissue. Results were statistically analyzed by 1-factor ANOVA with Fisher's PLSD test.

Abbreviation: DL, detection limit.

*$P < .05$, †$P < .01$, ‡$P < .001$: *v* group A.

§$P < .01$, ‖$P < .001$: *v* group B.

CTN Clearance and Correlation With the Remaining Functional Renal Tissue on Day 10

The calculated CTN clearance (CTN_{Cl}) is shown in Table 1. CTN_{Cl} in groups B and C, respectively, was 26% and 33% lower than in group A. To determine whether the decrease of CTN_{Cl} correlated with the degree of NX, we calculated the correlation coefficient between CTN_{Cl} and the remaining functional areas. A positive and significant correlation was found ($r = .574$, $P < .0001$): CTN_{Cl} decreased with the loss of functional tissue (Fig 4).

DISCUSSION

We have described the development and characterization of a chronic renal failure model in the laboratory mouse. In contrast to bigger laboratory animals, the mouse is easier to manage (housing, feeding, and breeding) and has a short life span, enabling longitudinal studies of the course of a disorder. There is sufficient similarity in the development and physiology of divergent mammals that findings in one species are often qualitatively valid for other mammalian species, including man. In addition, the development of models of renal failure in different species improves generalization to the target.

In the present study, we used NX and/or arterial ligation to induce renal failure. Many other techniques have been reported to induce renal failure in animals. They include either surgical procedures such as ablation of part of the kidney tissue[2,12] or ligation of the arteries,[12,16] physical procedures using electrocoagulation[3] or cryosurgery,[21] or chemical procedures like administration of nephrotoxins via the bloodstream[22,23] or in the subjects' food.[15] Finally, there are spontaneous animal models of renal failure such as polycystic kidney disease.[24]

The surgical method of arterial ligation used in the present study has previously been used in rats,[16] dogs,[12] and other species such as sheep.[1] This nephrectomy model induces a decrease of functional renal mass entailing a uremic state. Some investigators argue that, due to differences in arterial supply, reproducibility is difficult to obtain with the ligation technique.[21] However, in most mice of our strain, a regular ramification pattern of the renal artery was found, with a subdivision into two branches; sometimes each of the two main branches divides into two smaller branches. The biological parameters (Table 1), microscopic findings (Table 2), and quantification of GCs (Tables 3, 4, and 5) illustrate the reproducibility of our model.

The GC pattern appears to correspond to characteristics found in man. As the end products of nitrogen metabolism and candidate uremic toxins,[24,25] GCs are primarily excreted by the kidney, but their accumulation in patients with renal failure is due not only to reduced clearance but also to an increased production, as suggested for GSA.[25] These observations are consistent with the present findings in group C, showing a fourfold increase of GSA levels in plasma, a twofold increase in urine, and almost a threefold increase in the brain compared with control values. The increased production may explain the increased plasma and brain GSA levels and high urinary GSA excretion notwithstanding the significant reduction of CTN_{Cl}.

The large increase of other GCs in plasma, like CTN, α-keto-δ-GVA, GBA, G, and MG, in both nephrectomized

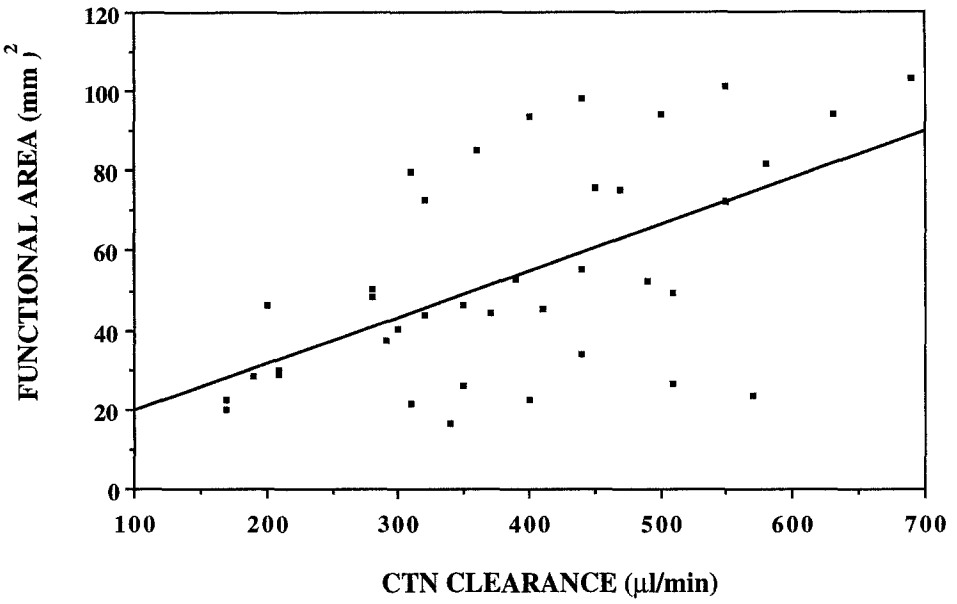

Fig 4. Functional area as a function of CTN_{Cl} in mice groups A, B, and C. Functional area is measured in left and right kidneys. Regression line formula: functional area = 0.08 + 1.17 (CTN_{Cl}). There is a positive and significant correlation between CTN_{Cl} and functional area ($r = .57$, $P < .0001$).

groups may reflect a defect in renal function due to renal injury. The most marked changes were observed in group C. The inefficiency of renal function could be caused by the severely reduced kidney mass and an overloading of the remnant nephrons.

Some GCs, such as GAA, showed a decreased plasma level. Urinary GAA excretion was even more pronounced, in agreement with results found in uremic rats.[16] Our results are also consistent with those found in a large group of nondialyzed patients with chronic renal failure.[26] GSA, CTN, G, and MG were increased in the cerebrospinal fluid[27] and in the evaluated brain structures of uremic patients.[28] The striking increase of G and GSA in the brain of our nephrectomized mice is concordant with findings in the brain of uremic patients[28] and rabbits.[29] It has been previously reported that renal failure may be accompanied by some neurological symptoms such as disorientation, confusion, a varying degree of uremic encephalopathy, and convulsions.[30] Several GCs have been suggested as possible uremic toxins. They have convulsive effects in experimental animals when administered systemically, intracisternally, or intraventricularly.[31-34] Several uremic GCs have been shown to block chloride channels, and GSA has been shown to act as an endogenous N-methyl-D-aspartate (NMDA)-type glutamate receptor agonist.[35,36]

The increase of brain GBA levels in our model was comparable to that previously observed in the brain of uremic rabbits.[29] GBA has a specific distribution related to the gamma-aminobutyric acid (GABA)ergic system.[37] Pisano et al[38] demonstrated that GBA is synthesized by transamination of arginine to GABA.

The results of our study show that renal failure induced in these mice disturbs GC metabolism and/or renal handling, leading to specific changes in plasma, urine, and brain levels. The model might be an interesting tool for further pathophysiological and behavioral investigations, in particular with regard to the role of different uremic toxins in the uremic syndrome.

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