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ORIGINAL ARTICLE

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# Influence of 72% injury in one kidney on several organs involved in guanidino compound metabolism: a time course study

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**Abstract** Arginine (Arg) produced from citrulline originates mostly from kidneys. Arg is involved in guanidino compound biosynthesis, which requires inter-organ co-operation. In renal insufficiency, citrulline accumulates in the plasma in proportion to renal damage. Thus, disturbances in Arg and guanidino compound metabolism are expected in several tissues. An original use of the model of nephrectomy based on ligating branches of the renal artery allowed us to investigate Arg and guanidino compound metabolism simultaneously in injured (left) and healthy (right) kidneys. The left kidney of adult rats was subjected to 72% nephrectomy. Non-operated, sham-operated and nephrectomized rats were studied for a period of 21 days. Constant renal growth was observed only in the healthy kidneys. Guanidino compound levels were modified transiently during the first 48 h. The metabolism and/or tissue content of several guanidino compounds were disturbed throughout the experimental period. Arg synthesis was greatly reduced in the injured kidney, while it increased in the healthy kidney. The renal production of guanidinoacetic acid decreased in the injured kidney and its urinary excretion was reduced. The experimentally proven toxins α-keto-δ-guanidinovaleric acid and guanidinosuccinic acid (GSA) accumulated only in the injured kidney. The urinary excretion of GSA and methylguanidine increased in nephrectomized rats.When the injured kidney grew again, the level of some guanidino compounds tended to normalize. Nephrectomy affected the guanidino compound levels and metabolism in muscles and liver. In conclusion, the specific accumulation of toxic guanidino compounds in the injured kidney reflects disturbances in renal metabolism and function. The healthy kidney compensates for the injured kidney's loss of metabolic functions (e.g. Arg: production). This model is excellent for investigating renal metabolism when a disease destroys a limited area in one kidney, as is observed in patients.

**Keywords** Acute and chronic renal failure · Arginine · Arginine-derived compounds · Nephrectomy · Liver · Muscle · Plasma · Time course study · Urine

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## Introduction

Arginine (Arg) is a key amino acid involved in several metabolic pathways including the production of nitric oxide (NO) [34], urea [21, 24], polyamines [40, 46], agmatine [29, 35] and guanidino compounds [45] such as guanidinoacetic acid (GAA), creatine (CT), creatinine (CTN) as well as methylated [18], acetylated, hydroxylated and oxidized Arg-derived compounds [30]. Several organs such as the small intestine, kidneys, liver and skeletal muscles contribute to Arg anabolism and catabolism. In the small intestine, glutamine is converted into successive nitrogenous products leading to citrulline (Cit) [53]. Cit is carried from the small intestine to the kidney by the blood without entering hepatocytes. The renal conversion of Cit to Arg has been extensively studied recently, and the renal sites of Arg synthesis precisely identified. The pars convoluta of the proximal tubule is the main site of Arg synthesis within the mammalian nephron [25]. In parallel to these microdissection studies, physiological studies performed in vivo on rats reveal that the Arg concentration in the renal venous blood is higher than that in the renal arterial blood [6, 13, 15]. The physiological importance of the renal production of Arg as well as the fate of Arg within the kidneys have been questioned intensely [14, 24, 42].

The kidneys contain several enzymes that metabolize Arg into GAA, CT and guanidinosuccinic acid (GSA) [15, 16, 19, 20, 42]. In addition, the intermediary compounds (e.g. GAA, CT) leave the kidney and are

carried through the bloodstream to specific tissues to be metabolized. Most GAA is methylated in the liver to form CT in vertebrate metabolism [5, 54]. CT is exported from the liver and transported by the blood to tissues that require it. Skeletal and cardiac muscles, the brain and a few other tissues contain a high creatine kinase activity (EC 2. 7. 3. 2), which catalyses the reversible transfer of the γ-phosphate group of ATP to the guanidino group of CT to yield ADP, $H^+$ and phosphoryl-creatine (PCr) [54]. PCr diffuses to the sites of ATP consumption. PCr and CT degradation is mostly a non-enzymatic reaction, leading to the cyclization of CT to CTN [54]. CTN, a non-ionic molecule, constantly diffuses out of the tissues and is either excreted in urine or metabolized to methylguanidine (MG) in the liver [36, 37, 41]. In addition to its role as a metabolic precursor, Arg released from the kidneys is essential for protein synthesis in extra-renal tissues [14].

The high level of the metabolic relationship and the interdependence between the kidneys, liver, small intestine and skeletal muscles strongly suggest that the partial or complete injury of one of these organs might dramatically disturb several metabolic pathways (e.g. Arg, polyamine and guanidino compound metabolism) not only in the injured organ, but also in organ(s) involved in at least one key step of their production. This statement is clearly supported by the following findings. Destroying renal tissue induces a stable and prolonged increase in citrullinemia in proportion to the degree of damage [28] and a decrease in GAA synthesis in mice [1], patients [32] and rabbits [22, 52] with chronic renal failure. Bilateral nephrectomy modified Arg, CT and GSA metabolism in liver perfused for 48 h following surgery [42]. Moreover, hepatectomy decreases citrullinemia [15]. Most of the publications concerning renal-insufficient animals and patients generally only report changes in plasma and urine guanidino compound levels [2, 12, 26, 32, 48]. Only a few papers have dealt with the determination of guanidino compounds in cerebrospinal fluid, brain [10, 11, 55] and liver [42]. However, the repercussions of renal damage on guanidino compound metabolism in each kidney, in the liver and muscles together with guanidino compound variations in plasma and urine remain unknown.

Therefore, we studied the influence of renal damage in one kidney upon renal, hepatic and muscular guanidino compound metabolism. Moreover, we hypothesized that Arg and guanidino compound metabolism could differ between a kidney submitted to 72% destruction by ligating branches of its renal arteries (ipsilateral; left kidney) and a healthy kidney (contralateral: right kidney). This protocol allowed us to investigate (1) the time course of changes in guanidino compound levels in the kidney, liver, skeletal muscle, plasma and urine; (2) guanidino compound levels during acute renal failure; (3) the influence of the contralateral kidney upon Arg and guanidino compound metabolism; and (4) inter-organ relationships in guanidino compound metabolism.

## Materials and methods

### Animals and induction of nephrectomy

Male Sprague Dawley rats (Iffa Credo, L'Arbresle sur Orge, France) initially weighing 190–220 g were used for these experiments. Animal experimentation was conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The rats were subdivided into nine groups. Group I: non-operated rats; group II: sham-operated rats; and groups III–IX: rats submitted to nephrectomy by ligating branches of the left renal artery in order to reduce its functional mass by ≅72%. The degree of necrosis was estimated by the visual method described previously [28]. The rats were anaesthetized i.p. with 0.1 ml Nembutal (6%) per 100 g body weight (BW) before the operation.

The rats were housed individually (12 h/12 h dark-light cycle) and had free access to tap water and standard laboratory food (Souffirat, Genthon, France). Before euthanasia, most of the rats were individually housed in a metabolic cage for an adaptative period of 3 days. Rats had again free access to tap water and standard laboratory food in the form of powder (Souffirat, Genthon, France). Urines were collected for 24 h before euthanasia.

### Tissue and biological fluid samples

Nephrectomized rats were killed 1, 2, 4, 7, 11, 16 or 21 days after nephrectomy. After anaesthesia, i.p. with 0.1 ml Nembutal per 100 g BW, rats were weighed. Samples of arterial blood were taken from the abdominal aorta to measure the plasma concentration of urea and guanidino compounds including CTN. The blood was transferred into lithium heparinized vacutainer tubes and maintained at 4°C. The right kidney and the non-ischaemic parts of the left kidney were removed, decapsulated, weighed and frozen separately on dry-ice. Samples of liver and gastrocnemius muscle were removed and frozen on dry-ice. The tissue samples were maintained at –50°C before determining guanidino compound levels. Blood samples were centrifuged at 4000 *g* for 20 min at 4°C and a fraction of the plasma was stored at –50°C until analysis.

The volume of the 24-h urine collection was measured. Urines were centrifuged at 4000 *g* for 10 min and samples of urine were frozen at –50°C until determination of guanidino compound concentrations. Although the tubular secretion of CTN is more marked in uremic animals and patients than in controls [23, 44], we determined the urinary CTN concentration (together with the plasma CTN levels) to calculate the CTN clearance and to estimate the glomerular filtration rate.

### Determination of biochemical compounds

The urinary CTN concentration was determined using an Astra 8 Beckman analyser. In tissues and plasma, urea nitrogen was determined with diacetylmonoxime as described by Ceriotti [7].

Samples of liver and muscle tissue and each whole kidney were homogenized separately in 1 ml water (2 ml for the whole kidney) in an ice bath with a "tissue tearor" (Biospec Products, Bartlesville, USA), model 985. The probe was washed immediately with 1 ml of a 300 g/l trichloroacetic acid solution at 4°C and added to the homogenate, which resulted in a protein precipitation after vortex mixing. After centrifugation (100,000 *g* for 30 min at 6°C) the clear supernatant was used for guanidino compound analysis.

The concentration of the guanidino compounds was determined using a Biotronic LC 5001 (Biotronik, Maintal, Germany) amino acid analyser adapted for guanidino compound determination. The guanidino compounds were separated over a cation exchange column using sodium citrate buffers and were detected with the fluorescence ninhydrin, method is reported in detail elsewhere [31].

**Table 1** Biological parameters in non-operated, sham-operated and nephrectomized rats. Results are expressed as means ± SE. ($Cl_{CTN}$ Creatinine clearance, *LK* left kidney, *RK* right kidney.) Results were analysed by ANOVA 1 and 2 factors and the Fisher PLSD test

| Treatment | None | Sham | Nephrectomized rats | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Experimental groups | I | II | III | IV | V | VI | VII | VIII | IX |
| Time after operation (days) | – | 1 | 1 | 2 | 4 | 7 | 11 | 16 | 21 |
| Number of rats | 12 | 11 | 5 | 5 | 5 | 5 | 5 | 6 | 5 |
| Body weight (g) | 223±4 | 223±4 | 204±3[c] | 205±8[c] | 225±6 | 255±4[c] | 277±4[c] | 351±13[c] | 382±8[c] |
| Damages in LK (%) | 0 | 0 | 71.8±0.6 | 74.7±3.0 | 70.1±1.5 | 73.2±3.0 | 70.5±1.0 | 69.6±2.5 | 75.3±2.0 |
| RK weight (mg) | 914±17 | 812±14[a] | 837±26 | 883±44 | 1008±17[c] | 1115±25[c] | 1256±48[c] | 1570±49[c] | 1631±63[c] |
| LK weight (mg) | 921±18 | 834±18[a] | 300±31[c] | 324±31[c] | 342±32[c] | 315±25[c] | 336±52[c] | 449±26[c] | 422±38[c] |
| Renal mass (mg) | 1835±33 | 1646±30[a] | 1137±28[c] | 1207±70[c] | 1349±49[a] | 1429±34[c] | 1593±37[a] | 2018±47[c] | 2053±88[c] |
| Urine volume (ml/day) | 8.2±0.6 | 8.03±0.41 | 9.4±2.7 | 14.2±2.1[c] | 24.8±2.9[c] | 18.3±0.9[c] | 17.9±1.2[c] | 23.8±3.3[c] | 20.0±1.1[c] |
| $Cl_{CTN}$ (ml/min) | 2.26±0.15 | 1.75±0.15[a] | 0.78±0.07[c] | 1.21±0.14[c] | 2.01±0.13 | 1.94±0.06 | 2.25±0.08[b] | 2.85±0.16[c] | 2.91±0.11[c] |

[a] Differs from non-operated rats; [c] differs from both control and sham-operated rats at the level $P<0.05$
[b] differs from sham-operated rats and

### Chemicals

Standard guanidino compounds were purchased from Sigma (St. Louis, Mo., USA), CT and CTN from Merck (Darmstadt, Germany). α-keto-δ-Guanidinovaleric acid was synthesized enzymatically as described earlier [31]. All other chemicals used were obtained from Merck and were of analytical grade.

### Calculations and statistics

Results are presented as means ± SE (standard error of the mean). Tissue urea and guanidino compound concentrations are expressed in mmol or µmol per gram wet weight, respectively, whereas those of plasma are given in mmol or µmol per liter. In urine samples, guanidino compounds are expressed in µmol per 24 h. Statistically significant differences were calculated by analysis of variance (ANOVA one and two factors, Statview II SE) and the Fisher PLSD test at the 95% level significance where appropriate. For correlation analyses, the correlation coefficient "*r*" was calculated using Cricket graph version 1.3.2 and "*P*" was determined from tables at the 95% level significance.

## Results

### Biological parameters

The degree of renal damage in the left kidney (72.2±1.0%) was similar in the seven groups of nephrectomized rats (Table 1). Non-operated and sham-operated rats had a similar body weight. In contrast, nephrectomized rats lost weight after the first few days following renal injury, but gained weight from day 7 until day 21. The weight of the right kidney increased linearly throughout the experimental period of 3 weeks ($r$=0.959, $P<0.001$) whereas that of the injured kidney did not increase except on days 16 and 21 ($P<0.001$). Destroying 72% of the left kidney provoked an inhibition of its growth for 2 weeks, but had no inhibitory effect on the right kidney (Fig.1). Urine volume was unaffected by nephrectomy (Table 1), but was significantly enhanced from the 2nd to the 21st day ($P<0.001$). Nephrectomy induced a concomitant and dramatic decrease in CTN clearance, because the number of functional glomeruli was reduced (Table 1). Consequently, the remaining glomeruli developed a hyperfiltration.

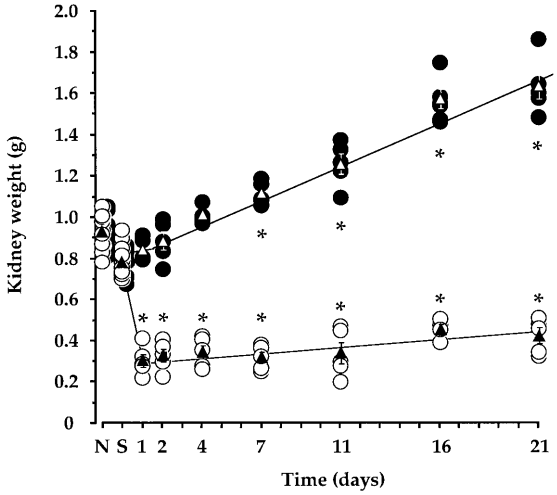


**Fig. 1** Time course of changes in kidney weight. *Symbols* represent either the individual value of the right (*closed circles*) and left kidney (*open circles*) or means ± SE for each group: right kidney (*open triangles* and *bars*) and left kidney (*closed triangles* and *bars*) obtained from non-operated (*N*), sham-operated (*S*) and nephrectomized rats. ANOVA one and two factors and Fisher PLSD test. *Different from non-operated and sham-operated rats

### Urea and creatinine

The uremia was not influenced by sham-operation, but peaked 24 h after nephrectomy and returned to high normal values within 4 days (Fig. 2; $P<0.0001$). Thereafter, the uremia increased slowly to quite constant levels, which differed from those of non- and sham-operated rats. In each kidney, the urea level tended to be enhanced after nephrectomy, but the increase did not reach statistical significance (Fig. 2). In skeletal muscle ($P<0.003$) and in liver ($P<0.0004$), the urea concentration peaked 24–48 h after nephrectomy before returning to the values observed in non-operated rats. However, in the liver, changes in the urea level more closely resembled those observed in plasma (Fig. 2).

Creatininemia was enhanced biphasically: the first peak was observed 24–48 h after nephrectomy, and the

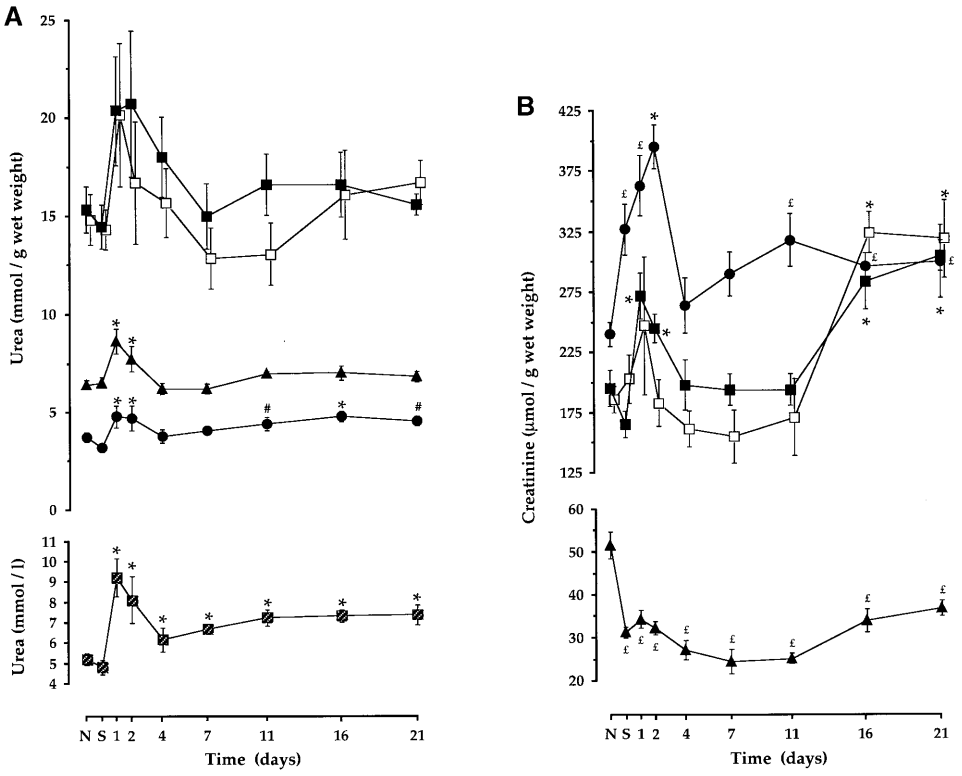


**Fig. 2** Time course of changes in tissue and plasma urea (**A**) and creatinine (**B**) levels. Results are given as means ± SE. Symbols represent – *closed* and *open squares*: right and left kidney, respectively; *closed triangles*: liver; *closed circles*: skeletal muscle and *hatched squares*: plasma. ANOVA one and two factors and Fisher PLSD test. *Different from non-operated (*N*) and sham-operated (*S*) rats, £different from non-operated rats, and #different from sham-operated rats. (*CTN* Creatinine)

second peak occurred on the 7th day and stayed constant until the 21st day [28]. The time course of changes in CTN in the healthy kidney was similar to that in the injured kidneys, and was enhanced biphasically (Fig. 2). Significantly higher CTN levels were observed 24–48 h after nephrectomy in the right kidney ($P<0.0001$) compared to non- and sham-operated rats. This was not observed in the left kidney. The renal CTN content increased on the 16th and the 21st days ($P<0.0001$) (Fig. 2). In skeletal muscle, the CTN level increased after the sham-operation, peaked on the 2nd day ($P<0.003$) and differed from that of the non-operated rats between days 11 and 21 (Fig. 2). Sham-operated and nephrectomized rats had significantly lower and constant hepatic CTN levels compared to non-operated rats (Fig. 2; $P<0.0001$).

## Guanidino compounds

### *Major changes in guanidino compound levels after nephrectomy*

*Arginine.* Changes in Arg levels are depicted in Fig. 3. Argininemia was higher in sham-operated than in non-operated rats. Twenty-four hours after nephrectomy, there was a remarkable transient decrease in argininemia. Forty-eight hours after nephrectomy, argininemia had raised above the basal level and remained quite stable from day 2 to day 21 ($P<0.0001$). In nephrectomized rats, the hepatic Arg content decreased 24–48 h after the surgery ($P<0.0003$) and returned to the basal level until day 21. In skeletal muscle, the Arg levels followed the same profile as in plasma, but, after nephrectomy, the Arg levels did not differ from the values measured in non-operated rats except on days 4 and 7 ($P<0.0045$). One of the most important findings was observed in kidneys. Renal Arg levels were similar in non-operated and sham-operated rats. In contrast, in nephrectomized rats, statistical analyses (2-way ANOVA) indicated that Arg levels in the left kidney differed dramatically from those in the right kidney ($F=74.9$; $P<0.0001$) and were influenced by nephrectomy ($F=10.8$; $P<0.0001$). In the injured kidney, the Arg concentration fell after nephrectomy, remained unchanged until day 11, but increased from day 16 to day 21 ($P<0.0001$). In contrast, the Arg content in the healthy kidney dramatically increased throughout the experimental period ($P<0.0001$). An increase in Arg synthesis in the right kidney might be related to the progressive increase in citrullinemia [28]. The urinary excretion of Arg was decreased 24 h after nephrectomy, but, thereafter, it increased in parallel to CTN clearance ($F=17$, $P<0.0001$; Table 2).

*Guanidinoacetic acid.* GAA results from Gly/Arg transamidination in the proximal tubule. Thus, changes in Arg levels might also alter GAA levels (Fig. 3). Quite similar variations in GAA levels were observed in liver, skeletal muscle and the plasma of non-operated, sham-operated and nephrectomized rats. In all tissues studied, the GAA level decreased 24 h after nephrectomy. Thereafter, plasma GAA levels increased to significantly higher levels from day 7 to day 21 ($P<0.0001$). In the liver, the GAA level returned to basal values within 48 h after

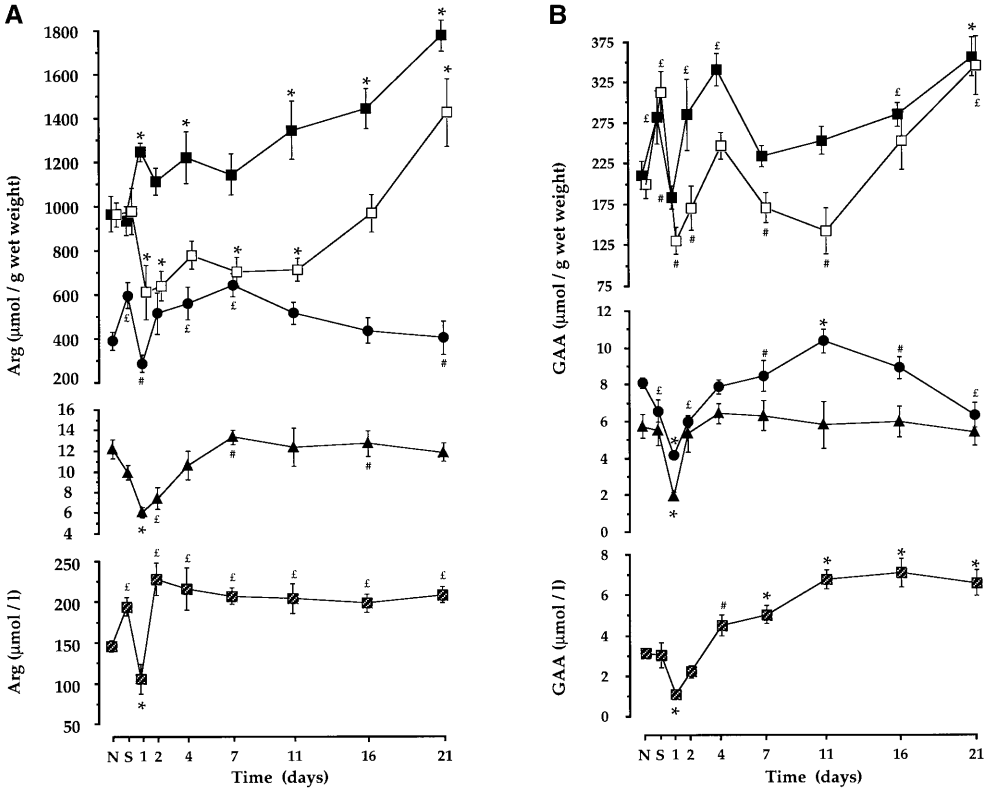


**Fig. 3** Time course of changes in tissue and plasma arginine (**A**) and guanidinoacetic acid (**B**) levels. Results are given as means ± SE. Symbols represent – *closed* and *open squares*: right and left kidney, respectively; *closed triangles*: liver; *closed circles*: skeletal muscle and *hatched squares*: plasma. ANOVA one and two factors and Fisher PLSD test. *Different from non-operated (*N*) and sham-operated (*S*) rats, £different from non-operated rats, and #different from sham-operated rats. (*Arg* Arginine, *GAA* guanidinoacetic acid)

nephrectomy. More or less the same result was observed for skeletal muscles. The healthy kidney exhibited higher GAA levels than the injured kidney (2-way ANOVA $F$=14.8; $P$<0.0002). In addition, nephrectomy influenced the variations in GAA content in both kidneys (2-way ANOVA $F$=9.8; $P$<0.0001). These results suggest that the high Arg levels measured in the right kidney might be responsible for a greater production of GAA in the right kidney compared to the left. GAA urinary excretion decreased after nephrectomy and remained below the levels observed in non- and sham-operated rats throughout the experimental period, suggesting a net reabsorption ($F$=14.03, $P$<0.0001; Fig. 7).

*Creatin.* The time course of changes in CT levels is depicted in Fig. 4. In all tissues, sham-operated rats had a higher CT level than non-operated rats. It seems that surgery induced a transient tissular production and/or accumulation of CT. Twenty-four hours after nephrectomy, tissue and plasma CT levels were sharply enhanced ($P$<0.0001 in all cases) and rapidly returned to basal values in kidneys, liver and plasma. In each kidney, the CT level was not significantly different (2-way ANOVA $F$=1.78, $P$=0.18). In the skeletal muscle of non-operated rats, the CT content was about 35- to 180-fold higher than in the previous tissues. In nephrectomized rats, the muscle CT content remained higher than the basal values ($P$<0.0001). CT urinary excretion was characterized by a dramatic peak, which occurred after sham-operation but was more pronounced after nephrectomy. One week later, CT urinary excretion in nephrectomized rats returned to the basal level ($F$=7.42, $P$<0.0001; Table 2 and Fig. 7).

*Guanidinosuccinic acid.* GSA tissue levels were not influenced by sham-operation except in the right kidney. In all tissues studied, nephrectomy induced a peak of GSA for 24–48 h. In plasma, GSA was enhanced by 373% and returned thereafter to almost basal values ($P$<0.0001). In the liver, GSA increased during the last 10 days of the experimental period (+195%, $P$<0.0001). No GSA could be detected in skeletal muscle; therefore, the levels must be at least lower than those found in the other tissues studied. Renal GSA content varied between the levels measured in the healthy and injured kidneys (2-way ANOVA, $F$=21.7, $P$<0.0001). During the follow-up study, the injured kidney contained higher GSA levels than the healthy one. In addition, nephrectomy was responsible for the first peak of GSA, which occurred during the acute phase of renal failure, and the second peak was only observed in the left kidney (2-way ANOVA, $F$=7, $P$<0.0001). GSA urinary excretion was increased only in nephrectomized rats. In urine samples, two peaks were observed: the first one was found 24 h after nephrectomy and the second one occurred on day 16 ($F$=4.46, $P$<0.0007; Table 2 and Fig. 7).

*β-guanidinopropionic acid.* Plasma β-GPA peaked on day 1 and returned rapidly to the basal value (Fig. 5;$P$<0.0001). In kidneys, nephrectomy induced significant variations in the time course of β-GPA changes (2-way ANOVA, $F$=2.1, $P$<0.0001). Sham-operated rats

**Table 2** Time course of changes in urinary excretion of guanidino compounds in non-operated, sham-operated and 72% nephrectomized rats. Results (means ± SE) are expressed in µmol guanidino compound excreted per day. (*ND* Not detectable, *NX* nephrectomy.) For other abbreviations, please see the text

| Experimental groups | I | II | III | IV | V | VI | VII | VIII | IX |
|---|---|---|---|---|---|---|---|---|---|
| Time after NX (days) | – | – | 1 | 2 | 4 | 7 | 11 | 16 | 21 |
| Number of rats | 6 | 6 | 4 | 5 | 5 | 5 | 5 | 6 | 5 |
| α-keto-δ-GVA | 1.44±0.19 | 0.69±0.08* | 0.36±0.03* | 0.77±0.13* | 1.21±0.20 | 1.54±0.24 | 1.37±0.14 | 1.85±0.20 | 1.98±0.17* |
| GSA | 0.145±0.004 | 0.146±0.023 | 0.228±0.027* | 0.161±0.009 | 0.162±0.007 | 0.186±0.009 | 0.185±0.012 | 0.394±0.028* | 0.227±0.054* |
| CT | 1.88±0.31 | 22.45±14.17 | 53.94±7.16* | 71.7±22.7* | 19.86±8.25 | 1.10±0.11 | 0.78±0.06 | 1.19±0.07 | 1.48±0.17 |
| GAA | 11.95±0.90 | 5.50±0.74* | 3.06±0.29* | 4.30±0.64* | 9.65±0.99* | 6.73±0.80* | 4.39±0.45* | 6.35±0.53* | 6.44±1.06* |
| α-N-AA | 1.49±0.25 | 0.86±0.16* | 0.52±0.05* | 0.69±0.07* | 1.16±0.26 | 0.99±0.16 | 0.94±0.14* | 1.47±0.14 | 1.46±0.25 |
| ArgA | 0.066±0.006 | 0.060±0.006 | 0.028±0.003* | 0.052±0.006 | 0.068±0.004 | 0.095±0.008* | 0.079±0.006 | 0.117±0.013* | 0.123±0.010* |
| β-GPA | 0.023±0.002 | 0.063±0.014* | 0.051±0.008* | 0.075±0.007* | 0.045±0.009 | 0.041±0.003 | 0.032±0.002 | 0.049±0.004* | 0.086±0.012* |
| γ-GBA | 5.16±0.26 | 2.68±0.47* | 1.10±0.03* | 1.81±0.17* | 3.86±0.31* | 4.45±0.32 | 4.70±0.45 | 6.44±0.49* | 6.99±0.37* |
| Arg | 1.47±0.19 | 0.70±0.10* | 0.42±0.03* | 0.70±0.10* | 1.52±0.15 | 1.37±0.18 | 1.38±0.16 | 2.14±0.18* | 2.23±0.19* |
| HArg | 0.035±0.003 | 0.024±0.004 | 0.014±0.003* | ND | 0.037±0.006 | 0.044±0.008 | 0.043±0.004 | 0.059±0.006* | 0.090±0.013* |
| G | 0.865±0.053 | 0.564±0.094* | 0.169±0.009* | 0.311±0.046* | 0.701±0.044 | 0.917±0.039 | 0.801±0.043 | 1.213±0.109* | 1.262±0.05* |
| MG | 1.628±0.119 | 1.877±0.405 | 0.291±0.028* | 0.887±0.195 | 2.57±0.15* | 3.20±0.10* | 2.97±0.19* | 4.21±0.42* | 4.36±0.29* |

Statistical significant differences were calculated by analysis of variance (ANOVA one factor, Statview II SE) and Fisher PLSD test at the level $P<0.05$. For legible reasons, only differences between the control group (I) and the other groups are reported (*)

had higher renal β-GPA levels than non-operated rats. In the right kidney, β-GPA increased 48 h after nephrectomy and then decreased to reach about half the basal level ($P<0.0001$). In the left kidney, the β-GPA level decreased rapidly below the basal values ($P<0.0017$). In the liver, β-GPA peaked 24–48 h after nephrectomy and decreased below the basal values on days 11 and 16 ($P<0.0001$). No important fluctuations in muscle β-GPA levels were observed. The β-GPA level was higher in sham-operated rats and lower in rats killed 4 days after nephrectomy compared to that of the non-operated rats ($P<0.0025$). β-GPA urinary excretion was higher in sham-operated and nephrectomized rats than in non-operated rats. An increase in β-GPA urinary excretion took place on days 2, 16 and 21 ($F=6.07$, $P<0.0001$; Table 2).

*γ-guanidinobutyric acid.* Sham-operation and nephrectomy induced a transient decrease in γ-GBA concentration in plasma, liver and kidneys ($P<0.0001$; Fig. 5). Thereafter, γ-GBA levels returned more or less rapidly to control values. There were no statistical differences in γ-GBA content between the two kidneys (2-way ANOVA, $F=1.58$, $P=0.21$). In the skeletal muscle of nephrectomized rats, γ-GBA levels were higher than the values found in sham-operated rats ($P<0.0001$). γ-GBA urinary excretion decreased 24 h after nephrectomy, but thereafter increased in parallel to CTN clearance ($F=30$, $P<0.0001$; Table 2).

*Homoarginine*. In general, homoarginine (HArg) levels were not deeply affected by nephrectomy as shown in Fig. 6. In plasma, HArg levels changed little after nephrectomy, as seen on days 1, 4 and 11 ($P<0.0001$). In the liver, HArg decreased 24–48 h after nephrectomy ($P<0.0049$) before returning to quite basal levels. HArg concentration did not differ between the kidneys ($F=0.425$; $P=0.516$), but nephrectomy might be responsible for variations in HArg level on days 1 and 4 in the right kidney ($F=5.06$; $P<0.0001$). In skeletal muscle, the HArg level decreased below the control value on day 1 and increased above the control level on day 7 ($P<0.0001$). HArg urinary excretion decreased after nephrectomy, but thereafter, while it did increase in parallel with CTN clearance, the amount of HArg excreted did not differ from that of the non-operated rats except on days 16 and 21 ($F=13.6$, $P<0.0001$; Table 2).

*α-keto-δ-guanidinovaleric acid.* A very interesting result was observed by measuring α-keto-δ-guanidinovaleric acid (α-keto-δ-GVA) levels in kidneys (Fig. 6). Statistical analyses (2-way ANOVA) indicated that the α-keto-δ-GVA level differed between the kidneys ($F=81$; $P<0.0001$) and was influenced by nephrectomy ($F=9.38$; $P<0.0001$). In the injured kidney, α-keto-δ-GVA accumulated after nephrectomy and remained at high levels before decreasing on day 21 ($P<0.0001$). In contrast, sham-operation only slightly decreased α-keto-δ-GVA

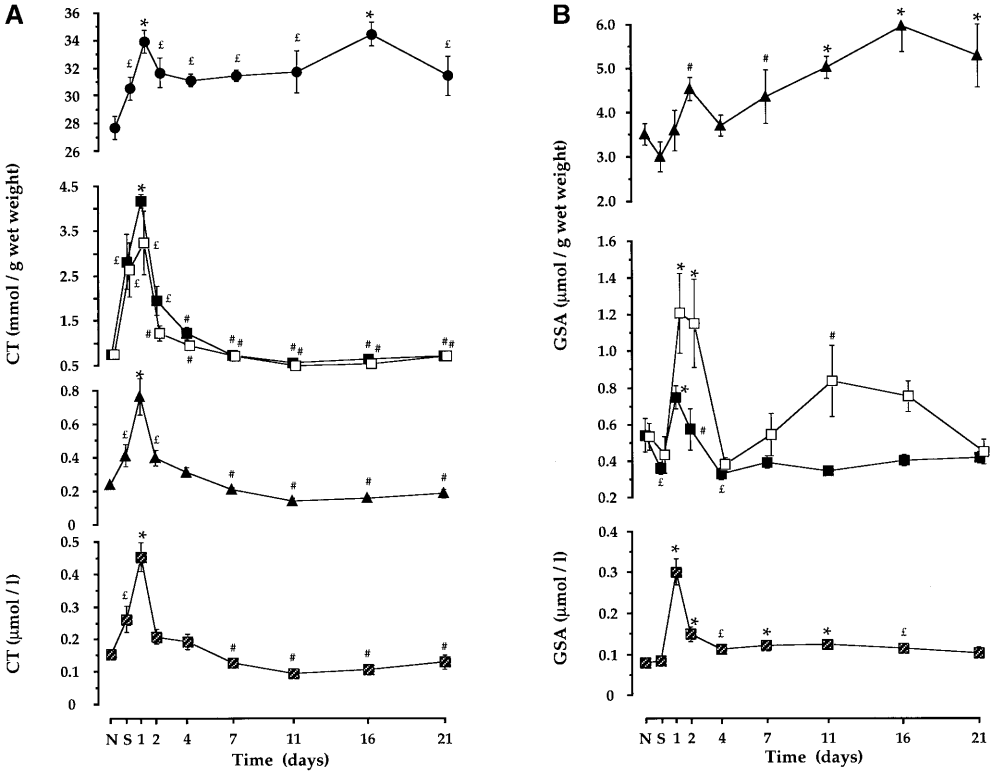


**Fig. 4** Time course of changes in tissue and plasma creatine (**A**) and guanidinosuccinic acid (**B**) levels. Results are given as means ± SE. Symbols represent – *closed* and *open squares*: right and left kidney, respectively; *closed triangles*: liver; *closed circles*: skeletal muscle and *hatched squares*: plasma. ANOVA one and two factors and Fisher PLSD test. *Different from non-operated (*N*) and sham-operated (*S*) rats, £different from non-operated rats, and #different from sham-operated rats. (*CT* Creatine, *GSA* guanidinosuccinic acid)

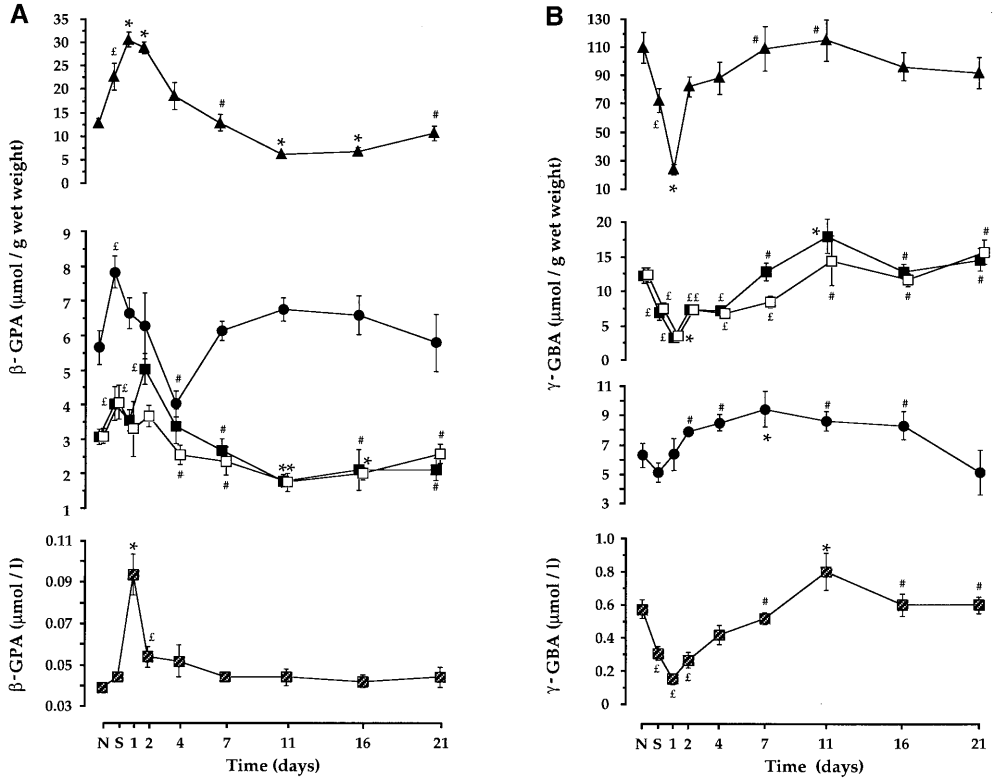


**Fig. 5** Time course of changes in tissue and plasma β-guanidinopropionic acid (**A**) and γ-guanidinobutyric acid (**B**) levels. Results are given as means ± SE. Symbols represent – *closed* and *open squares*: right and left kidney, respectively; *closed triangles*: liver; *closed circles*: skeletal muscle and *hatched squares*: plasma. ANOVA one and two factors and Fisher PLSD test. *Different from non-operated (*N*) and sham-operated (*S*) rats, £different from non-operated rats, and #different from sham-operated rats. (*β-GPA* β-Guanidinopropionic acid, *γ-GBA* γ-guanidinobutyric acid)

levels in the healthy kidney, and in nephrectomized rats α-keto-δ-GVA levels were similar to those of non-operated rats. Concerning the other tissues (Fig. 6), α-keto-δ-GVA could not be detected in skeletal muscle. In the liver, the α-keto-δ-GVA concentration tended to increase after nephrectomy, as seen on days 7, 16 and 21 ($P<0.0002$). The time course of urinary excretion of α-keto-δ-GVA resembled that of HArg very closely ($F=10.7$, $P<0.0001$; Table 2).

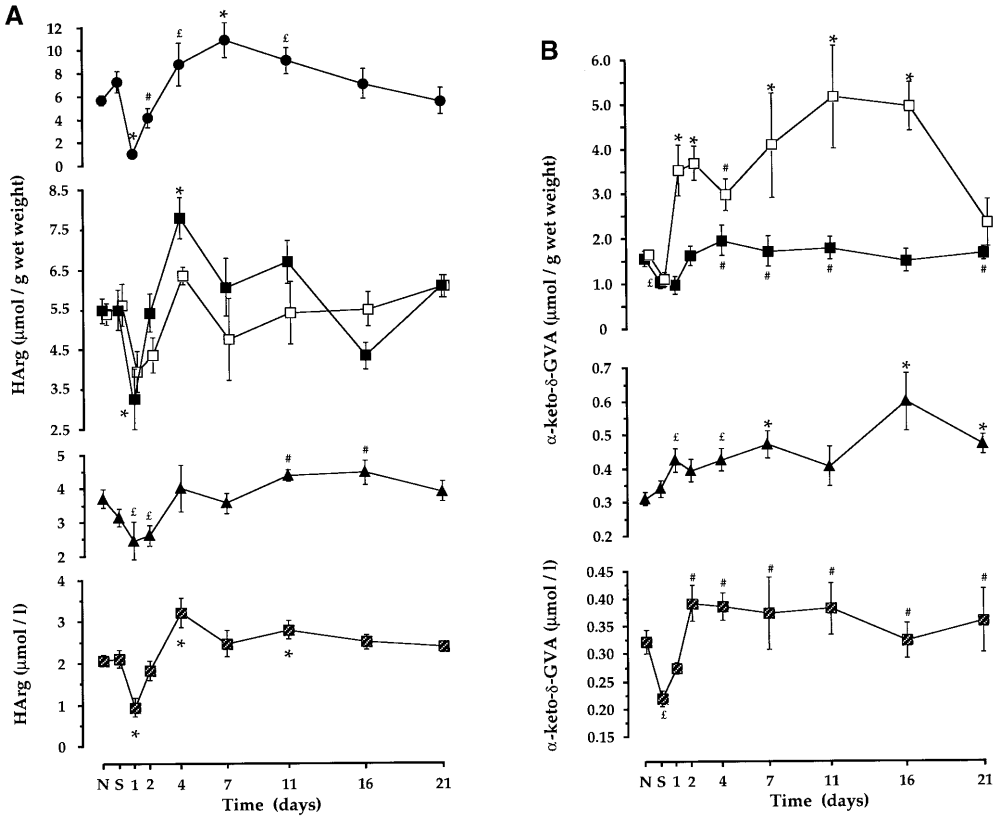


**Fig. 6** Time course of changes in tissue and plasma homoarginine (**A**) and α-keto-δ-guanidinovaleric acid (**B**) levels. Results are given as means ± SE. Symbols represent – *closed* and *open squares*: right and left kidney, respectively; *closed triangles*: liver; *closed circles*: skeletal muscle and *hatched squares*: plasma. ANOVA one and two factors and Fisher PLSD test. *Different from non-operated (*N*) and sham-operated (*S*) rats, £different from non-operated rats, and #different from sham-operated rats. (*HArg* Homoarginine, *α-keto-δ-GVA* α-keto-δ-guanidinovaleric acid)

*Minor or no changes in guanidino compound levels after nephrectomy*

*Argininic acid.* In plasma, but not in skeletal muscle and the liver of non-operated and nephrectomized rats, the ArgA levels were lower than the detection limit. The levels in the kidney could not be determined for analytical reasons. In skeletal muscle, nephrectomy decreased the ArgA level only on days 2 and 4; thereafter no changes were observed (data not shown). In contrast, in the liver, the ArgA level tended to increase progressively from day 4 until day 21 ($P<0.0001$). The time course of urinary excretion of ArgA resembled to that of HArg ($F=16.1$, $P<0.0001$; Table 2).

*α-N-acetylarginine.* In plasma, the low level of α-*N*-acetylarginine (α-N-AA) was comparable in non- and sham-operated rats. The α-N-AA level increased after nephrectomy (data not shown). In the liver, α-N-AA content was not influenced by nephrectomy (data not shown). α-N-AA was undetectable in kidneys and the skeletal muscle. α-N-AA urinary excretion in nephrectomized rats was below the basal level from day 1 to day 11 and, thereafter, did not differ from that of non-operated rats ($F=3.84$, $P<0.0001$; Table 2).

*Guanidine.* Guanidine was detected in the different tissues. Nephrectomy did not significantly change the guanidine content of skeletal muscle and kidneys (data not shown). In the liver, the guanidine concentration was slightly decreased after sham-operation and nephrectomy ($P<0.047$, data not shown). In plasma, although the guanidine level was decreased after sham-operation, its level increased above the basal value in nephrectomized rats ($P<0.0318$; data not shown). The time course of urinary guanidine excretion closely resembled that of HArg ($F=30.4$, $P<0.0001$; Table 2).

*Methylguanidine.* Sham-operation and nephrectomy did modify methylguanidine (MG) concentrations in the plasma, liver, skeletal muscle and kidneys (data not shown). MG urinary excretion was decreased by nephrectomy, but thereafter it increased in parallel to CTN clearance and reached a level 3 times higher than that of the basal values ($F=27.7$ $P<0.0001$; Table 2 and Fig. 7).

Urinary guanidino compound excretion

Nephrectomy induced rapidly a decrease in CTN clearance, which reflects a reduction of the glomerular filtration rate (Table 1 and [28]). As a consequence, urinary guanidino compound excretion was diminished and might contribute to the enhanced plasma and tissue guanidino compound levels observed during the acute period following nephrectomy (Table 2). Thereafter, during the later period (day 4 to day 21), the urine volume increased in nephrectomized rats and calculations indicate that CTN clearance progressively increased with time. Close positive correlations between changes in

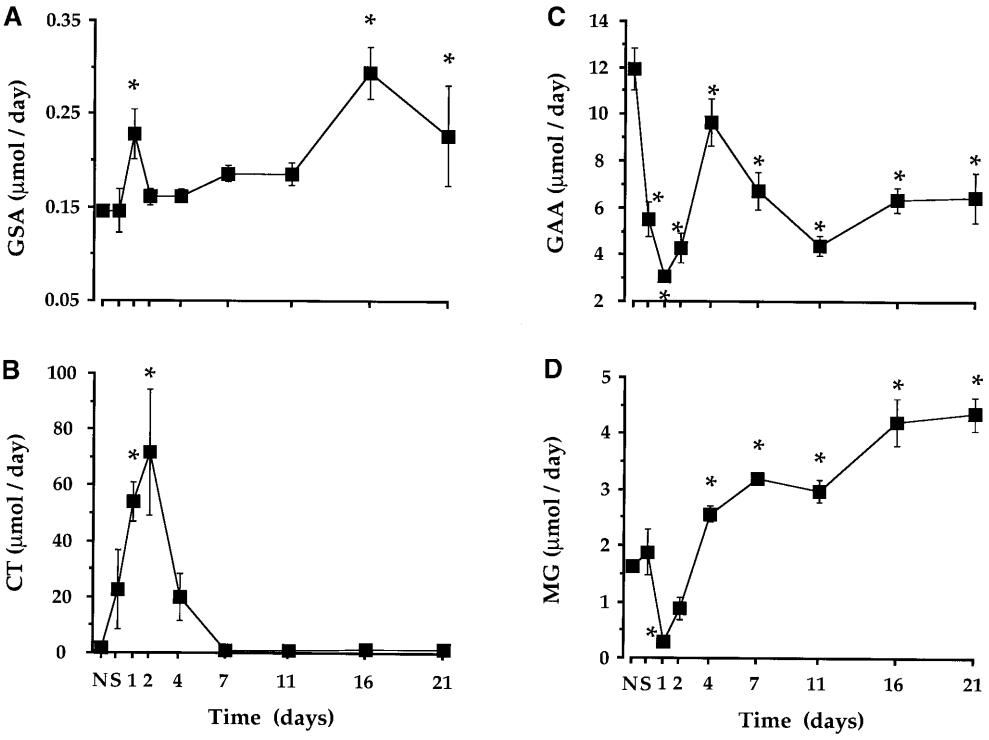


**Fig. 7** Time course of urinary excretion of guanidinosuccinic acid (**A**), creatine (**B**), guanidinoacetic acid (**C**) and methylguanidine (**D**). For each rat, urine was collected for 24 h and results are expressed in µmoles guanidino compound excreted per day. Each *closed square* represents the mean ± SE. ANOVA one and two factor and Fisher PLSD test. *Significantly different from non-operated rats (*N*). (*CT* Creatine, *GAA* guanidinoacetic acid, *GSA* guanidinosuccinic acid, *MG* methylguanidine)

**Table 3** Correlation between guanidino compound urinary excretion and creatinine clearance. For statistical analyses, the correlation coefficient "*r*" was calculated and *P* was determined from a table of a handbook at the 0.01 level of significance (*)

| Guanidino compounds | *r* |
|---|---|
| α-keto-δ-GVA | 0.9397* |
| GSA | 0.3549 |
| GAA | 0.4472 |
| α-N-AA | 0.9116* |
| ArgA | 0.8775* |
| β-GPA | 0.0031 |
| γ-GBA | 0.9798* |
| Arg | 0.8118* |
| HArg | 0.9066* |
| G | 0.7810* |
| MG | 0.9148* |

CTN clearance (as an index of the glomerular filtration rate) and the daily amount of guanidino compound excreted in urine are shown in Table 3. The data indicate that there was a urinary leakage of the following nitrogenous compounds: α-keto-δ-GVA, α-N-AA, ArgA, γ-GBA, Arg, HArg, guanidine and MG (Table 2). Finally, an increase in urine excretion facilitated the elimination of "uremic toxins" and this physiological adaptation might be partly responsible for the decrease in plasma and tissue guanidino compound levels during the later period of the experiment.

## Discussion

Although modifications in guanidino compound metabolism have been documented in uremic patients and animals affected by various degrees of renal insufficiency [2, 12, 26, 32, 48], no experiment was performed on animals to study the consequences of destroying a large part of only one kidney on guanidino compound metabolism simultaneously in the injured kidney, the contralateral kidney, which remains intact, and other organs. Because the kidney is the main site of Arg production [14], the tissue accumulation or depletion of several Arg-derived compounds as well as modifications in their metabolism were expected in uremic animals. To achieve this work, an experimental nephrectomy was induced by destroying 72% of the mass of the left kidney and guanidino compounds were measured over a time course study of 21 days. Ligation of the appropriate renal arteries of the left kidney provoked a deep reduction in the number of functional glomeruli and nephrons (≅72%) resulting in a dramatic decrease in tubular enzyme activities and impairing renal metabolism and the urinary excretion of organic compounds. As a consequence of tissue destruction, important variations in guanidino compound levels are expected during the acute phase of renal failure. Moreover, during the progression of chronic renal failure, a combination of physiological and metabolic adaptations or non-adaptations might be responsible for the increase in single-nephron glomerular filtration rate, changes in renal enzyme activities, the accumulation of "uremic toxins" and variations in tissue guanidino compound levels.

The most important variations in tissue guanidino compound levels and the inter-connections between the metabolic pathways distributed in the different tissues are described and summarized below.

## The metabolic route of arginine, guanidinoacetic acid and creatine

In the kidney, the proximal tubule contains a great variety of enzymes metabolizing amino acids including Arg, which plays a pivotal role in several metabolic pathways. Arg synthesis depends on the citrulline concentration, it requires aspartate and takes place essentially in the proximal convoluted tubules [13, 25]. In kidneys, the highest level of Arg is located in the cortex followed by the outer stripe of the outer medulla – two zones where the proximal tubules lie [27]. In animals submitted to experimental nephrectomy, destroying the proximal tubules results in an enhancement of citrullinemia and an accumulation of citrulline in extra-renal organs in proportion to renal damage [8, 28, 49]. In parallel, Arg synthase activity is decreased in the kidneys of uremic rats [8]. These data suggest that the capacity of Arg production in the injured kidney is reduced.

In the present study, the Arg levels in healthy kidneys were higher in nephrectomized rats compared to control- and sham-operated rats, while the injured kidneys contained less Arg except when the rats began to gain weight again. This important result strongly supports that Arg production was reduced in the injured kidney because of a decrease in Arg synthase activity and a reduction in the number of functional proximal tubules. In contrast, in the healthy kidney, the increase in citrulline availability caused by the enhancement of citrullinemia might favour Arg synthesis [28]. At present, it remains unknown whether the protein levels of argininosuccinate synthetase (EC: 6.3.4.5) and argininosuccinate lyase (EC: 4.3.2.1) were modified in each kidney.

Variations in renal Arg content are very important since, in the presence of glycine, Arg is converted into GAA by glycine-arginine transamidinase (EC: 2.1.4.1). This metabolic step occurs not only in proximal convoluted tubules, but also in pancreatic cells and in hepatocytes [33, 43, 47, 50]. In our study, the availability of Arg is relatively well correlated with variations in renal GAA content, since their levels varied in parallel. Indeed, higher Arg and GAA concentrations were observed in the healthy kidney compared to the injured kidney. In addition, between day 11 and day 21 (Fig. 3) the Arg and GAA levels increased simultaneously. In uremic animals and patients, the low renal production and tissue concentration of GAA are associated with a sharp decrease in the urinary excretion of GAA (Fig. 7) [1, 22, 32, 52]. However, an adaptive reabsorption of GAA might prevent its leakage into urine [26]. At present, it remains unknown whether the glycine-arginine transamidinase activity was inhibited in uremic animals.

The production of GAA is important because its methylation leads to the production of CT, a potent energetic compound when phosphorylated. Even though the liver is the major site of CT synthesis, the kidneys also produce this compound [19, 20].

## The production and accumulation of uremic toxins: guanidinosuccinic acid, α-keto-δ-guanidinovaleric acid and methylguanidine

The model of nephrectomy used was well adapted to demonstrate the specific accumulation of GSA and α-keto-δ-guanidinovaleric acid in the remaining functional portion of the injured kidney, but not in the contralateral healthy kidney. These two compounds are experimentally proven to be toxins. They are accumulated in the plasma, urine and tissues of uremic patients and animals [1, 11, 26, 32].

Guanidinosuccinic acid synthesis requires urea as a precursor and takes place in the liver [3]. Because the tissue urea concentration is enhanced in uremic patients and animals compared to healthy subjects, GSA synthesis might be favoured. In the healthy kidney, the small peak of GSA might be associated with the decrease in CTN clearance (Table 1) while in the injured kidney, it remains unclear why the remaining part of the kidney submitted to nephrectomy specifically accumulated high concentrations of GSA. It is questioned whether the injured kidney synthesizes more GSA or is unable to further metabolize it. Cohen et al. [9] proposed that an analogue enzyme of glycine-arginine transamidinase might produce GSA + ornithine from Arg and aspartate or, it may be that argininosuccinic acid is the precursor. In contrast, Natelson proposed the existence of a "urea-guanidine bi-cycle" to produce GSA [38, 39].

The oxidative deamination of Arg by L-amino-acid oxidase yields α-keto-δ-guanidinovaleric acid plus $NH_3$ and $H_2O_2$. In the healthy kidney, the α-keto-δ-guanidinovaleric acid level remained unchanged while a dramatically enhanced α-keto-δ-guanidinovaleric acid concentration was found in the injured kidney. Moreover, the regular increase in the urinary excretion of α-keto-δ-guanidinovaleric acid suggests that uremic rats overproduce this compound. It is questioned whether the remaining functional portion of the injured kidney produced more α-keto-δ-guanidinovaleric acid than the healthy kidney or whether it cannot further metabolize α-keto-δ-guanidinovaleric acid into γ-guanidinobutyric acid or ArgA. Indeed, in the presence of $H_2O_2$, α-keto-δ-guanidinovaleric acid is converted chemically into γ-guanidinobutyric acid [51]. Alternatively, α-keto-δ-GVA can be converted into ArgA, but since the latter could not be measured in the kidney for analytical reasons, it remains unclear whether this route is used in the kidneys of uremic rats. In addition, the local production of $H_2O_2$ in these metabolic steps might be responsible for the additional renal damage.

Methylguanidine is another well-known uremic toxin synthesized from CTN in hepatocytes [36, 37, 41, 56]. In our experiments, it seems that the degree of nephrectomy is insufficient to observe the accumulation of MG in tissue. However, since MG urinary excretion increased constantly (Fig. 7), this compound might be overproduced and regularly eliminated from the body. In animals and patients with a high degree of renal failure,

MG accumulates in the plasma, urine and tissues [1, 4, 11, 17, 26, 32].

Because guanidino compound synthesis involves inter-organ cooperation, the partial destruction of one kidney might provoke alteration of the guanidino compound concentration in the plasma, kidneys, liver and muscles. Most of the changes occurred during the acute phase following nephrectomy.

In conclusion, the injured kidney specifically accumulates GSA and α-keto-δ-guanidinovaleric acid, two uremic toxins, and reduces its production of Arg and GAA, while the healthy kidney enhanced the synthesis of these latter two compounds. Tissue-specific patterns of guanidino compound concentrations were observed.

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## References

1. Al Banchaabouchi M, Marescau B, D'Hooge R, VanMarck E, VanDaele A, Levillain O, De Deyn PP (1998) Biochemical and histopathological changes in nephrectomized mice. Metabolism 47:355–361
2. Anderstam B, Katzarski K, Bergstrom J (1997) Serum levels of *N*-$^{G}$,*N*-$^{G}$-dimethyl-L-arginine, a potential endogenous nitric oxide inhibitor in dialysis patients. J Am Soc Nephrol 8: 1437–1442
3. Aoyagi K, Ohba S, Narita M, Tojo S (1983) Regulation of biosynthesis of guanidinosuccinic acid in isolated rat hepatocytes and in vivo. Kidney Int. 24:S224–S228
4. Barsotti G, Bevilacqua G, Morelli E, Cappelli P, Balestri PL, Giovannetti S (1975) Toxicity arising from guanidine compounds: role of methylguanidine as a uremic toxin. Kidney Int 7:S299–S301
5. Borsook H, Dubnoff JW (1941) The formation of glycocyamine in animal tissues. J Biol Chem 138:389–403
6. Bouby N, Hassler C, Parvy P, Bankir L (1993) Renal synthesis of arginine in chronic renal failure: in vivo and in vitro studies in rats with 5/6 nephrectomy. Kidney Int 44:676–683
7. Ceriotti G (1971) Ultramicro determination of plasma urea by reaction with diacetylmonoxime antipyrine without deproteinization. Clin Chem 17:400–402
8. Chan W, Wang M, Kopple JD, Swendseid ME (1974) Citrulline levels and urea cycle enzymes in uremic rats. J Nutr 104: 678–683
9. Cohen BD, Stein IM, Bonas JE (1968) Guanidinosuccinic aciduria in uremia. A possible alternate pathway for urea synthesis. Am J Med 45:63–68
10. De Deyn PP, Marescau B, Cuykens JJ, Gorp LV, 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
11. 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
12. De Deyn PP, Robitaille P, Vanasse M, Qureshi IA, Marescau B (1995) Serum guanidino compound levels in uremic pediatric patients treated with hemodialysis or continuous cycle peritoneal dialysis. Nephron 69:411–417
13. Dhanakoti SN, Brosnan JT, Herzberg GR, Brosnan ME (1990) Renal arginine synthesis:studies in vitro and in vivo. Am J Physiol 259:E437–E442
14. Featherston WR, Rogers QR, Freedland RA (1973) Relative importance of kidney and liver in synthesis of arginine by the rat. Am J Physiol 224:127–129
15. Funahashi M, Kato H, Shiosaka S, Nakagawa H (1981) Formation of arginine and guanidinoacetic acid in the kidney in vivo. Their relations with the liver and their regulation. J Biochem 89:1347–1356
16. Gerber GB, Koszalka TR, Gerber G, Altman KI (1962) Biosynthesis of creatine by the kidney. Nature 196:286–287
17. Giovannetti S, Balestri PL, Barsotti G (1973) Methylguanidine in uremia. Arch Intern Med 131:709–713
18. Kakimoto Y, Akazawa S (1970) Isolation and identification of $N^{G}$,$N^{G}$-and $N^{G}$,$N'^{G}$-dimethyl-L-arginine, *N*-ε-mono-, di, and trimethyllysine, and glucosylgalactosyl- and galactosyl-δ-hydroxylysine from human urine. J Biol Chem 245:5751–5758
19. Koszalka TR (1967) Extrahepatic creatine synthesis in the rat. Role of the pancreas and kidney. Arch Biochem Biophys 122: 400–405
20. Koszalka TR, Bauman AN (1966) Biosynthesis of creatine by the perfused rat kidney. Nature 212:691–693
21. Krebs HA, Henseleit K (1932) Untersuchungen über die Harnstoffbildung im Tierköper. Z Physiol Chem 210:33–66
22. Kuroda M (1993) Study on impaired metabolism of guanidinoacetic acid in chronic renal failure rabbits with special reference to impaired conversion of arginine to guanidinoacetic acid. Nephron 65:605–611
23. Levey AS, Perrone RD, Madias NE (1988) Serum creatinine and renal function. Annu Rev Med 39:465–490
24. Levillain O, Hus-Citharel A (1996) Distribution of arginase along the mammalian nephron. In: De Deyn PP, Marescau B, Qureshi IA, Mori A (eds) Guanidino compounds in medicine and biology, vol. 2. Libbey, London, pp 77–85
25. Levillain O, Hus-Citharel A, Morel F, Bankir L (1990) Localization of arginine synthesis along rat nephron. Am J Physiol 259:F916–F923
26. Levillain O, Marescau B, De Deyn PP (1995) Guanidino compound metabolism in rats subjected to 20% to 90% nephrectomy. Kidney Int 47:464–472
27. Levillain O, Marescau B, De Deyn PP (1997) Renal handling of guanidino compounds in rat and rabbit. J Physiol (Lond) 499:561–570
28. Levillain O, Parvy P, Hassler C (1997) Amino acid handling in uremic rats: citrulline, a reliable marker of renal insufficiency and proximal tubular dysfunction. Metabolism 46:611–618
29. Lortie MJ, Novotny WF, Peterson OW, Vallon V, Malvey K, Mendonca M, Satriano J, Insel P, Thomson SC, Blantz RC (1996) Agmatine, a bioactive metabolite of arginine – production, degradation, and functional effects in the kidney of the rat. J Clin Invest 97:413–420
30. Marescau B, De Deyn PP, Lowenthal A, Qureshi IA, Antonozzi I, Bachmann C, Cederbaum SD, Cerone R, Chamoles N, Colombo JP, Hyland K, Gatti R, Kang SS, Letarte J, Lambert M, Mizutani N, Possemiers I, Rezvani I, Snyderman SE, Terheggen HG, Yoshino M (1990) Guanidino compound analysis as a complementary diagnostic parameter for hyperargininemia: follow-up of guanidino compound levels during therapy. Pediatr Res 27:297–303
31. Marescau B, Deshmukh 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
32. Marescau B, Nagels G, Possemiers I, De Broe ME, Becaus 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
33. McGuire DM, Gross MD, Elde P, Van Pilsum JF (1986) Localization of L-arginine-glycine amidinotransferase protein in rat tissues by immunofluorescence microscopy. J Histochem Cytochem 34:429–435

34. Moncada S, Palmer RMJ, Higgs EA (1989) Biosynthesis of nitric oxide from L-arginine. Biochem Pharmacol 38: 1709–1715
35. Morrissey J, McCracken R, Ishidoya S, Klahr S (1995) Partial cloning and characterization of an arginine decarboxylase in the kidney. Kidney Int 47:1458–1461
36. Nagase S, Aoyagi K, Narita M, Tojo S (1985) Biosynthesis of methylguanidine in isolated rat hepatocytes and in vivo. Nephron 40:470–475
37. Nagase S, Aoyagi K, Sakamoto M, Takemura K, Ishikawa T, Narita M (1992) Biosynthesis of methylguanidine in the hepatic microsomal fraction. Nephron 62:182–186
38. 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
39. Natelson S, Sherwin JE (1979) Proposed mechanism for urea nitrogen re-utilization relationship between urea and proposed guanidine cycles. Clin Chem 25:1343–1344
40. Pegg AE, McCann PP (1982) Polyamine metabolism and function. Am J Physiol 243:C212–C221
41. Perez G, Faluotico R (1973) Creatinine: a precursor of methylguanidine. Experientia 29:1473–1474
42. Perez GO, Epstien M, Rietberg B, Loutzenhiser R (1978) Metabolism of arginine by the isolated perfused rat kidney. Am J Physiol 255:F376–F380
43. Perez GO, Rietberg B, Owens B, Schiff ER (1977) Effect of acute uremia on arginine metabolism and urea and guanidino acid production by perfused rat liver. Pflügers Arch 372: 275–278
44. Perrone RD, Madias NE, Levey AS (1992) Serum creatinine as an index of renal function: new insights into old concepts. Clin Chem 38:1933–1953
45. Robin Y (1982) Metabolism of arginine in invertebrates: relation to urea cycle to other guanidine derivates. In: Lowenthal A, Mori A, Marescau B (eds) Urea cycle diseases. Plenum, New York, pp 407–417
46. Seiler N (1990) Polyamine metabolism. Digestion 46:319–330
47. Sorenson RL, Stout LE, Brelje TC, Van Pilsum JF, McGuire DM (1995) Evidence for the role of pancreatic acinar cells in the production of ornithine and guanidinoacetic acid by L-arginine:glycine amidinotransferase. Pancreas 10:389–394
48. Stein IM, Perez G, Johnson R, Cummings NB (1971) Serum levels and urinary excretion of methylguanidine in chronic renal failure. J Lab Clin Med 77:1020–1024
49. Swendseid ME, Wang M, Chan W, Kopple JD (1975) The urea cycle in uremia. Kidney Int 7:S280–S284
50. Takeda M, Kiyatake I, Koide H, Jung KY, Endou H (1992) Biosynthesis of guanidinoacetic acid in isolated renal tubules. Eur J Clin Chem Clin Biochem 30:325–331
51. Thoai NV, Roche J (1960) Dérivés guanidiques biologiques. In: Zechmeister L (ed) Progress in the chemistry of organic natural products. Springer, Berlin Heidelberg New York, pp 83–121
52. Tofuku Y, Muramoto H, Kuroda M, Takeda R (1985) Impaired metabolism of guanidinoacetic acid in uremia. Nephron 41: 174–178
53. Windmueller HG, Spaeth AE (1980) Respiratory fuels and nitrogen metabolism in vivo in small intestine of fed rats. J Biol Chem 255:107–112
54. Wyss M, Kaddurah-Daouk R (2000) Creatine and creatinine metabolism. Physiol Rev 80:1107–1213
55. Yamamoto Y, Saito A, Manji T, Nishi H, Ito K, Maeda K, Ohta K, Kobayashi K (1978) A new automated analytical method for guanidino compounds and their cerebrospinal fluid levels in uremia. Trans Am Soc Artific Organs 24:61–68
56. Yokozawa T, Fujitsuka N, Oura H (1990) Production of methylguanidine from creatinine in normal rats and rats with renal failure. Nephron 56:249–254