Endogenous Regulators of NO Bioavailability in Rats with Acute Renal Failure

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We studied the impact of acute glycerol-induced renal failure on blood levels and daily urinary excretion of arginine, monomethylarginine, and asymmetric and symmetric dimethylarginine. Acute renal failure was accompanied by enhanced daily excretion of asymmetric and symmetric dimethylarginine, increased plasma level of symmetric dimethylarginine, and decreased plasma level of arginine. Reabsorption of arginine and its methylated analogues decreased, thus compensating for reduced glomerular filtration rate. These data attest to increased production of dimethylarginines during acute renal failure. These changes can decrease NO bioavailability.

Key Words: *arginine; methylarginine; nitric oxide; renal failure*

Amino acid arginine (Arg) and its methylated analogues (methylarginines) are essential for the regulation of NO bioavailability in the body. Arg is a substrate for intracellular enzyme NO-synthase. Monomethylarginine (MMA) and asymmetric dimethylarginine (ADMA) are the major endogenous inhibitors of NO-synthase. Symmetric dimethylarginine (SDMA) does not affect activity of NO-synthase, but competes with Arg for transmembrane transporters of basic amino acids, thus limiting bioavailability of the substrate [1,4,11,13].

Arg enters the body with food and is synthesized in some organs [4]. Methylarginines are formed during methylation of arginine residues in nuclear proteins and released as a result of proteolysis [1,13]. The synthesis of Arg and methylarginines, their reabsorption, and excretion occur in the kidney, which plays a crucial role in the metabolism of Arg and its methylated derivatives [4,6]. In addition, high level of dimethylarginine dimethylaminohydrolase (DDAH) has been found in the kidney. This enzyme hydrolyzes ADMA and MMA providing the main route of their elimination [6,9]. SDMA is not subjected to enzymatic degradation and is excreted unchanged with the urine. Increased plasma levels of ADMA in patients with chronic renal failure can influence NO synthesis [15] and is associated with the development of endothelial dysfunction, early atherosclerosis, hypertension, and other cardiovascular diseases [11].

Here we studied the impact of acute renal failure (ARF) on the metabolism of Arg and methylarginines. For this purpose, we measured blood levels and excretion of Arg and methylarginines in rats with glycerol-induced ARF. ARF is a polyetiological disease, which is manifested by potentially reversible renal dysfunction. Despite the lack of published data on methylarginines during ARF, we hypothesized that this pathology can affect metabolism of Arg and its methylated analogues.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 250±5 g in accordance with the Directives of the European Community (86/609/EC) and approved by the Biomedical Ethics Committee (Institute of Physiology, Siberian Division of the Russian

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Academy of Medical Sciences). The animals were kept in individual metabolic cages with free access to water and food.

ARF (n=16) was induced by injection of 50% aqueous solution of glycerol into hind limb muscles (10 ml/kg body weight) [11]. The animals were deprived of water 24 h before injection. Immediately after the administration of glycerol, access to water was restored. The animals of the reference group (n=8) received the same volume of 0.9% NaCl. The animals were sacrificed by decapitation under ether anesthesia in 24 h (n=6) and 72 h (n=18) after the injection. At the same time points, daily urine volume was recorded; blood and urea samples were collected.

Arg and methylarginines were assayed by HPLC with fluorescence detection after solid phase extraction [2]. Creatinine and urea concentrations in the samples were assessed by the kinetic method in a clinical laboratory. Creatinine Arg and methylarginine clearance was calculated by the formula: $X_{CL}=U_X \times V/P_X$, where X_{CL} is clearance of substance X (ml/min); U_X is the concentration of X in the urine; P_X is plasma concentration of X; and V is urine volume (ml). Glomerular filtration rate (GFR) was assumed to be equal to creatinine clearance. Excreted fractions of Arg and methylarginines were determined by the formula $EF_X=X_{CL} \times 100/GFR$, where X_{CL} is clearance of substance X (ml/min).

The data were processed statistically using Statistica 10 software. The significance of differences between the means was evaluated by Kruskal–Wallis and ANOVA followed by post-hoc comparisons. The significance of differences was 0.01. Cluster analysis was conducted using the k-means algorithm. Spearman's correlation analysis was performed for the entire sample.

RESULTS

Intramuscular injection of glycerol leads to muscle necrosis and the release of myoglobin into the blood. Pathological changes in the kidneys under conditions of glycerol model are similar to those during ARF in humans with rhabdomyolysis and/or intravascular hemolysis [12].

Impaired excretory kidney functions, *i.e.* reduced GFR, uremia, creatinemia were reported after glycerol injection. However, no significant differences were revealed by these parameters in 24 h and 72 h after glycerol injection. On the basis of cluster analysis results, the animals with ARF were divided into two clusters according to GFR values: moderate (mean GFR 33.3±8.3% of control) and severe (mean GFR 4.2±3.3% of control) kidney damage (Table 1).

The data on urinary excretion of Arg and methylarginines are presented in Table 2 and their plasma levels in Table 3. ARF was characterized by pronounced (>100-fold) increase in daily excretion of ADMA. At the same time, plasma level of ADMA did not change significantly. The excretion of SDMA also increased, but only in animals with moderate kidney damage (cluster 1). ARF increased plasma level of SDMA. Correlation analysis revealed a strong relationship between SDMA and blood indicators of renal excretory function: GFR (r=-0.86, p<0.01), creatinine (r=0.94, p<0.01), and plasma urea (r=0.94, p<0.01). The observed relationship between blood SDMA and indicators of renal function suggest that blood plasma SDMA is an early marker of GFR changes in experimental and clinical practice, which is consistent with the previously reported data [6].

Our results suggest that ARF increases daily production of methylarginines (ADMA and SDMA). ARF is associated with increased methylation of arginine residues in proteins and/or proteolysis of dimethylarginine-containing proteins. Increased expression of protein-arginine methyltransferases (enzymes engaged in protein methylation of Arg) was shown in other kidney diseases [8]. Increased ADMA in daily urine observed by us can be associated with not only its enhanced production, but also impaired enzymatic elimination. DDAH can be readily oxidized and lose its enzymatic activity [8]. Intrarenal inflammation, oxidative stress associated with uremia, and uremic toxins reduce activity of DDAH [8,9]. As a consequence, a portion of ADMA normally subjected to enzymatic hydrolysis, is excreted unchanged in the urine during ARF. Increased methylation and/or proteolysis and decreased DDAH

TABLE 1. Main Indicators of Renal Excretory Function in the Model of Glycerol-Induced ARF ($M \pm SD$)

Indicator	Control (n=8)	$ARF_{GFR>0.1}$ (n=5)	$ARF_{GFR<0.1}$ (n=11)
GFR, ml/min	1.2±0.2	0.4±0.1*	0.05±0.04*+
Plasma creatinine, μM	37.5±6.5	75.2±17.4*	266.6±70.7*+
Plasma urea, mM	7.2±1.9	23.0±13.5*	51.4±13.1*+
Water reabsorption, %	99.4±0.1	96.5±0.7*	85.6±9.4*+

Note. Here and in Tables 2, 3: p<0.01 in comparison with *control, $^{+}ARF_{GFR>0.1}$.

TABLE 2. Urinary Excretion of Arginine and Methylarginines and Their Excreted Fractions over 24 h (*M*±*SD*)

Indicator		Control (*n*=8)	$ARF_{GFR>0.1}$ (*n*=5)	$ARF_{GFR<0.1}$ (*n*=11)
Daily urinary excretion, nM/24 h	Arg	1301.7±584.7	3026.2±1970.1	1255.0±781.3
	ADMA	0.13±0.27	109.1±62.6*	24.4±35.3*+
	SDMA	157.4±50.5	340.5±80.9*	122.1±96.2
	MMA	25.7±11.2	33.3±8.5	19.7±16.8
Excreted fraction, %	Arg	0.43±0.26	3.0±2.8*	16.2±13.1*+
	ADMA	0.01±0.03	29.2±27.8*	51.4±45.1*
	SDMA	35.8±9.3	70.8±18.2*	78.4±48.7*
	MMA	2.5±0.6	16.0±10.6*	49.5±33.0*+

activity did not affect MMA blood and urea levels. Since MMA is a precursor of ADMA and SDMA [6], changed metabolism of these dimethylarginines could probably influence MMA metabolism, but this was not observed.

ARF had no effect on daily excretion of Arg. However, in animals with severe kidney damage (cluster 2), plasma level of Arg was reduced (Table 3) and Arg/plasma ADMA ratio decreased. This NO-synthase substrate/inhibitor ratio reflects NO bioavailability. Reduced Arg/ADMA is associated with a decrease in blood flow through the kidneys, liver, and spleen [10]. In our study, we found a positive correlation between GFR and Arg/ADMA values (r=0.60, p<0.01). GFR strictly depends on the intensity of blood flow through the glomerulus. Thus, according to our data, preglomerular vasoconstriction and decreased NO production by renal glomeruli that are characteristic of ARF glycerol model [3,14] can be a result of limited Arg availability for NO-synthase of the renal vascular endothelium. This restriction is associated with decreased Arg synthesis in damaged nephron tubules and enhanced Arg absorption by the liver and other organs [5].

In our study, ARF was accompanied by decreased water reabsorption (Table 1). Reabsorption of all analyzed substances was also reduced, because their excreted fractions (Table 2) increased and correlated with the fraction of reabsorbed water (r=-0.93, r=-0.83, r=-0.74, and r=-0.92 for Arg, ADMA, SDMA, and MMA respectively; p<0.01). The highest excretion was recorded in animals with severe kidney damage. Reduced reabsorption of Arg and methylarginines was probably associated with the destruction of the proximal tubules [12], where amino acids are normally reabsorbed. In our experiment, daily excretion of Arg and its methylated derivatives was not below the control values. This was based on the increase in the proportion of the excretion of filtered substances compensating for the decline in daily filtrate. This phenomenon can be of both physiological (compensatory) importance and result from impaired function of the nephron proximal tubules.

Thus, ARF affects the metabolism of endogenous NO regulators in different ways: the formation of SDMA and ADMA increased and enzymatic ADMA cleavage decreased and was compensated by increased urinary excretion. Severe damage of renal function (low GFR) decreased plasma level of Arg. These changes can limit NO bioavailability in the body.

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TABLE 3. Plasma Concentrations (μM) of Arginine and Methylarginines (*M*±*SD*)

Indicator	Control (*n*=8)	$ARF_{GFR>0.1}$ (*n*=5)	$ARF_{GFR<0.1}$ (*n*=11)
Arg	188.9±36.2	195.9±36.1	120.6±44.2*
ADMA	0.70±0.11	0.82±0.26	0.62±0.26
SDMA	0.27±0.07	0.84±0.31*	1.77±0.43*+
MMA	0.63±0.21	0.44±0.24	0.48±0.12
Arg/ADMA	276.6±68.5	245.0±29.5	202.4±34.8*

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