BIOPHYSICS AND BIOCHEMISTRY

Effect of a Blocker of Nitric Oxide Production on Albumin Excretion by Rat Kidney

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Experiments on Wistar rats showed that single intraperitoneal injection nonselective NO-synthase inhibitor L-NAME in a dose of 50 mg/kg was followed by transient proteinuria and albuminuria. This effect was not reproduced by injection of ODQ, an inhibitor of intracellular effects of NO, and arginine, but D-NAME, an optical isomer of L-NAME not blocking NO-synthase, produced similar, though less pronounced effect. The degree of proteinuria and albuminuria increased in combined treatment with nitroarginine methyl esters and 1-deamino-arginine vasotocin or arginine vasopressin. Proteinuria during treatment with arginine derivatives attests to not only their effect on the charge of the filtration membrane, but also the participation of NO-dependent processes in the regulation of ultrafiltration in renal glomeruli.

Key Words: *proteinuria; albuminuria; nitric oxide; D-NAME; L-NAME*

Proteinuria, and first of all albuminuria, is one of the most common disturbances of renal functions [3]. Glomerular filter is a natural barrier preventing protein penetration into the nephron lumen; filtered proteins are then reabsorbed in the proximal segment of the nephron [10]. Normally, proteins are practically absent in human and animal urine [10]; however, our recent studies demonstrated transient proteinuria in healthy rats in different variants of diuresis stimulation [2] and the mechanism of this phenomenon is unknown. This physiological proteinuria can be determined by increased penetration of proteins through the glomerular filter. It is known that glomerular hemodynamics is controlled by NO-dependent processes [8], while glomerular filter permeability for proteins is largely determined by the negative charge of its molecules [10]. Hence, modulation of the charge of the glomerular filter and NO production can result in the appearance of proteins, *e.g.* albumins, in the urine.

Here we studied the effect of NO synthesis blockade with L-nitroarginine methyl ester (L-NAME) [9] on excretion of albumins by rat kidney.

MATERIALS AND METHODS

Experiments were carried out on female Wistar rats weighing 150-220 g. L-NAME and D-NAME in a dose of 50 mg/kg and L-arginine in a dose of 32 mg/kg in physiological saline were injected intraperitoneally. ODQ was dissolved in a small volume of DMSO, diluted with physiological saline, and injected intraperitoneally in a dose of 2 mg/kg. 1-Deamino-arginine vasotocin (1d-AVT) or arginine vasopressin (AVP) in a dose of 0.5 μg/kg in 1 ml/kg physiological saline or the same volume of physiological saline without the preparations were injected intramuscularly 15 min after administration of L-NAME, D-NAME, or ODQ. Experimental series with injection of 1d-AVT and AVP

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served as the control. The study protocol was approved by Ethical Committee of Institute of Evolution Physiology and Biochemistry, Russian Academy of Sciences.

The animals were placed in tight box cages with wire floor. Spontaneous diuresis was measured over 4 h. The volume of the urine was determined, creatinine concentration was measured by the kinetic method (Jaffe reaction); total protein content was assayed on an EOS Bravo W automated biochemical analyzer using pyrogallol reagent. Urine albumin was measured by immunoenzyme assay on an ELx800 automated reader (BIO-TEK Instruments) using Bethyl Laboratories kits. Vasotocin analogue 1d-AVT was synthesized by Peptide Synthesis Company [4]. L-NAME, D-NAME, L-arginine, AVP, and ODQ were purchased from Sigma-Aldrich.

Differences of the studied parameters from the control values were evaluated by ANOVA Kruskal—Wallis test and Dunn test [1] for multiple comparisons, the differences were significant at $p<0.05$.

RESULTS

Administration of L-NAME slightly increased diuresis, while excretion of protein increased 20-fold and excretion of albumin increased many times (Table 1). For evaluation of the contribution of NO synthesis blockade into proteinuria development, the effect of L-NAME was compared with that of D-NAME, an optical isomer of L-NAME not blocking NO-synthase, and ODQ, an inhibitor of soluble guanylate cyclase not preventing NO production, but blocking its effect at the level of cGMP synthesis [7,12]. Injection of 2 mg/kg ODQ had no effect of protein excretion. D-NAME 2-fold increased excretion of proteins (Table 1), whereas albumin excretion increased 32-fold compared to the control. Hence, acute proteinuria and albuminuria after administration of nitroarginine methyl esters are not the result of blockade of NO effects. Nevertheless, the role of NO in the regulation of albumin excretion cannot be excluded. It should be noted that despite similar effects of L-NAME and D-NAME on rat kidneys, L-NAME produced a 10-fold more potent stimulatory effect on protein excretion.

Administration of basic amino acids (arginine, lysine, ornithine) in high doses leads to tubular proteinuria due to suppression of proximal protein reabsorption [11] and L-NAME and D-NAME are arginine derivatives. Administration of L-arginine in a dose equimolar to the dose of L-NAME did not increase the excretion of protein and albumin by the kidney (Table 1). Evaluation of reversibility of L-NAME-induced changes in the renal barrier selectivity for proteins showed that high proteinuria was observed during the first 2 h after single parenteral administration of the preparation and then its severity decreased. One day after administration of L-NAME, protein excretion with the urine still 2-fold surpassed the control (3-fold for albumin) and on day 7 the parameters of protein excretion returned to the initial level (Fig. 1).

Single parenteral administration of nitroarginine methyl esters modulates selectivity of the glomerular filter for proteins, probably by affecting its charge, and leads to the development of acute selective proteinuria.

TABLE 1. Parameters of Renal Function in Rats (per 100 g body weight) after Intraperitoneal Injection of L-NAME, D-NAME, ODQ, or L-Arginine and under the Effect of 1d-AVT or AVP ($M\pm m$)

Experimental conditions	n	Excretion over 2 h			Creatinine clearance, ml/min
		urine, ml	total protein, μg	sodium, μg	
Control	10	0.36±0.31	143±75	5.8±1.3	0.27±0.13
L-arginine	10	0.14±0.04	118±28	3.8±1.7	0.22±0.04
L-NAME	9	0.82±0.44*	2800±2000*	1300±600*	0.23±0.10
D-NAME	10	0.36±0.17	284±174*	186±69*	0.16±0.03
ODQ	7	0.40±0.23	162±49	4.7±1.8	0.30±0.04
1d-AVT	10	1.54±0.55*	361±135*	20.8±4.2*	0.26±0.09
D-NAME+1d-AVT	10	1.07±0.23*	12370±11700*	11400±3800*	0.21±0.03
L-NAME+1d-AVT	9	1.44±0.47*	22100±11600*	24400±5400*	0.30±0.11
AVP	10	0.21±0.26	214±103*	no data	0.21±0.08
L-NAME+AVP	7	0.95±0.36*	8550±5070*	8090±5980*	0.18±0.03

Note. *$p<0.05$ compared to the control.

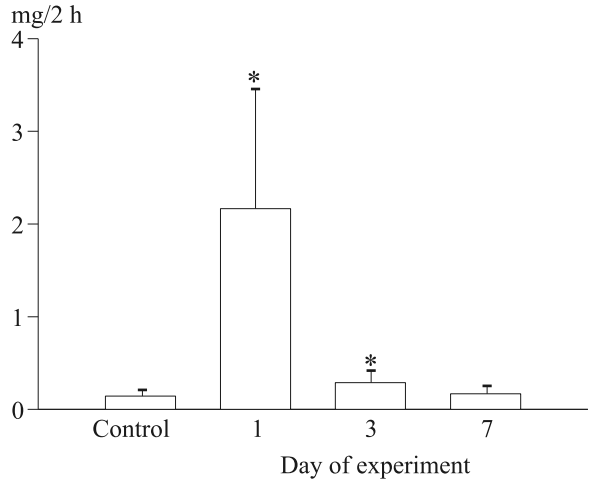

Fig. 1. Protein excretion by rat kidney over one week after L-NAME injection. *p<0.05 compared to the control.

It was interesting to study changes in proteinuria under conditions of polyuria, associated, according to our previous findings, with enhanced protein excretion [2]. Increased proteinuria and albuminuria observed after treatment with nitroarginine derivatives (1d-AVT; Table 1) were probably related to the increase in intraglomerular pressure. Similar changes were revealed in rats treated with AVP inducing a 3-fold increase in protein excretion compared to initial proteinuria (Table 1), but not causing polyuria.

Long-term blockade of NO production caused by L-NAME addition to drinking water induced proteinuria and albuminuria in experimental rats [5]. This effect was explained by the development of hypertension followed by damage to renal glomeruli in long-term NO-synthase blockade [6]. Here we first demonstrated that L-NAME practically immediately after its intraperitoneal injection sharply and considerably changed excretion of total protein and albumin in rats. This effect was not observed after injection of ODQ, but was present, though less pronounced after administration of D-NAME. These findings suggest that blockade of NO synthesis is not the leading factor in the development of L-NAME-dependent proteinuria. Proteinuria during treatment with arginine derivatives, compounds characterized by high positive charge, attests to not only their effect on the charge of the filtration membrane, but also participation of NO-dependent processes in the regulation of ultrafiltration in the renal glomeruli.

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